REMARKS

All references to page and line numbers of the specification are those found in PCT Publication No. WO 03/104479.

Amendments to the Claims

Applicants have amended the claims as indicated above in the Listing of the Claims.

In particular, Applicants have inserted the sequence identifier "(SEQ ID NO:2)" after the term "Nercc1 kinase protein" in Claim 1 to specify the amino acid sequence of the Nercc1 kinase protein and have inserted the sequence identifier "(SEQ ID NO:6)" after the term "Nek7" in Claim 8 to specify the amino acid sequence of the Nek7 kinase substrate. Support for the amendments is found in the Sequence Listing and the specification. See, e.g., page 23, lines 25-28, and page 23, line 33 - page 24, line 2, of the specification. Applicants have also amended Claim 1 to state that the term "kinase substrate" is "a polypeptide that comprises a domain that is susceptible to phosphorylation by said Nercc1 kinase protein" as taught in the specification. See, e.g., page 6, lines 14-16, of the specification. Accordingly, the amendments incorporate information disclosed in the specification and add no new matter.

Applicants have amended Claims 2 and 3 to delete the term "initially" as superfluous to the meaning of the claims and in accordance with the Examiner's recommendation. See, e.g., section 10, on page 7 of the Office Action dated May 8, 2007. Accordingly, the amendment adds no new matter.

Entry of the amendments to the claims is respectfully requested.

No amendment to the claims, withdrawal of claims, or cancellation of claims in this application should be interpreted as a surrender of subject matter by Applicants. Accordingly, Applicants reserve the right to pursue patent coverage in one or more continuation or divisional applications for any subject matter disclosed in a claim prior to any amendment, withdrawal, or cancellation thereof or disclosed in the specification of the instant application.

Restriction and Election of Species for Examination

Applicants affirm that the Examiner's restriction requirement with election of species for examination has been deemed final over Applicants' traverse. Accordingly, Applicants' provisional election to prosecute claims of Group 1 consisting of Claims 1-16 has been made final as has Applicants' election of the following species for examination of the claims of Group 1: a non-activated Nercc1 kinase as the species of Nercc1 kinase protein, a non-activated Nek7 kinase or fusion protein thereof as the species of kinase substrate, detection using an antibody as the species of method for determining the level of phosphorylated kinase substrate, a phosphorylated Nek7 kinase or fusion protein thereof as the species of phosphorylated kinase substrate, and a microtiter plate as the species of vessel in which at least one step of the claimed method is carried out. The Examiner has withdrawn Claims 4-7, 9, 10, and 17-45 as

being drawn to non-elected inventions. Claims 1-3, 8, and 11-16 were examined in the pending Office Action.

Compliance with Sequence Listing Requirement of 37 C.F.R. §§ 1.821 - 1.825

In section 5 of the Office Action, the Examiner objected to the specification as lacking compliance with the Sequence Listing Requirements of 37 C.F.R. §§ 1.821 - 1.825 in that the amino acid sequence presented in Figure 2A did not contain a sequence identifier. Applicants note that although Figure 2A does not contain a sequence identifier for the amino acid sequence recited in the figure, the sequence identifier "amino acids 891-940 of SEQ ID NO:2" is properly provided in the description of Figure 2A found in the Brief Description of the Drawings at page 9, lines 5-6 of the specification. With respect to location and format of a sequence identifier, the Manual of Patent Examining Procedure (MPEP) states:

> "It should be noted, though, that when a sequence is presented in a drawing, regardless of the format or the manner of presentation of that sequence in the drawing, the sequence must still be included in the Sequence Listing and the sequence identifier ("SEQ ID NO:X") must be used, either in the drawing or in the Brief Description of the Drawings." (last paragraph of MPEP § 2422.02; emphasis added)

In addition:

> ". . . In the situation where a contiguous fragment of a sequence that has already been properly set forth in a 'Sequence Listing' is discussed and/or claimed, the fragment does not need to be separately included in the 'Sequence Listing.' It may be referred to in the specification, claims or drawings as, e.g., 'residues 2 through 33 of SEQ ID NO:12,' assuming that SEQ ID NO:12 has been properly included in the 'Sequence Listing.' (last paragraph of MPEP § 2423.03; emphasis added)

The above excerpts from the MPEP clearly indicate that the location and format of the sequence identifier of the amino acid sequence recited in Figure 2A is proper and in compliance with the Sequence Listing Requirements for a U.S. patent application. Accordingly, reconsideration and withdrawal of the objection are respectfully requested.

Objection to Blank Spaces in the Specification and Cross-Reference to Related Applications

In section 6 of the Office Action, the Examiner objected to the presence of blank spaces from lines 1-7 on the first page of the specification:

> "The blank space from lines 1-7 must be deleted, because it cannot be determined if something was intended for the space in the application and is missing.
>
> "The identifying data of all prior applications for which benefits are claimed should be provided in either the first sentence(s) of the specification or in an application data sheet. See MPEP § 202.2" (section 6, page 5, of the Office Action)

Applicants acknowledge the absence of text on lines 1-7 on page 1 of the specification. So far as Applicants are aware, since there is no provision for eliminating blank spaces under 37 C.F.R. § 1.121(b), the avoidance of having to determine if something was intended for these blank spaces is not a basis for objecting to the specification. The specification was not objected to when received in the U.S. Receiving Office for the PCT; when formatted for publication as PCT Publication No. WO 03/104479, published December 18, 2003; when examined by the International Preliminary Examining Authority/U.S.; and, notably, when formatted by the U.S. Patent Office for publication as U.S. Publication No. 2006/0052582, published March 9, 2006. Accordingly, Applicants respectfully submit that the several blank lines above the title of the specification would not be confusing to persons skilled in this art to whom the specification is directed and that, based on the past history of successful formatting and publication of this specification, the formatting and printing programs of the U.S. Patent Office will be able to produce and publish any U.S. patent that issues based on this specification.

Applicants also preliminarily amended the specification to include the priority claim in a Cross-Reference to Related Applications. The Notice of Acceptance of Application Under 35 U.S.C. 371 and 37 C.F.R. § 1.495, dated November 9, 2005, specifically acknowledged timely completion of all requirements as of December 10, 2004 for entry into the U.S. national phase, including Applicants' preliminary amendment to insert the priority claim as submitted with this U.S. national stage application under 35 U.S.C. 371 on December 10, 2004. That preliminary amendment specifically directed that a Cross-Reference to Related Applications should be inserted after the title on page 1 of the specification. Again, there is no indication that the preliminary amendment is improper or would be confusing to the skilled practitioner in the art.

The above comments and documentation from the record show that the specification has been twice formatted for publication and has been deemed in full compliance with all requirements under 35 U.S.C. 371 and 37 C.F.R. § 1.495. Applicants respectfully submit that the specification is not confusing to the skilled practitioner and that Applicants have properly and timely amended the specification to include a cross-reference to prior applications in accordance with relevant statutes and regulations. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the objection of section 6, page 5 of the Office Action.

Rejections Under 35 U.S.C. 112, second paragraph

In section 8 of the Office Action, the Examiner rejected Claims 1-3, 8, and 11-16 under 35 U.S.C. 112, second paragraph, as indefinite for recitation of the term "lower level." In particular, the Examiner stated that the term "lower level" is a relative term that was not defined in the claim, that the specification does not provide a standard for ascertaining the requisite degree, that one of ordinary skill in the art would

not be reasonably apprised of the scope of the invention, and that one skilled in the art cannot determine *how much* lower the level of phosphoryated kinase substrate must be for the mitosis inhibitor to be identified. Applicants respectfully traverse the rejection for the reasons explained below.

Applicants' invention is based on the discovery that Nercc1 kinase-mediated phosphorylation of Nek6 or its homolog Nek7 is a critical step in a cascade of kinases that regulates eukaryotic cell entry into and maintenance of mitosis. See, e.g., page 4, lines 16-27; page 23, lines 19-21; Examples 10-13, pages 53-69; of the specification. According to the invention, since Nercc1 kinase-mediated phosphorylation is a critical activity in regulating mitosis, *any* lowering of the level of phosphorylation produced by Nercc1 kinase in the presence of an inhibitory compound compared to the level produced in the absence of the compound is sufficient to identify the compound as an inhibitor of mitosis. The Examiner is correct that the term "a lower level" is a relative term, but the Examiner is incorrect that the term is not defined in Claim 1. Claim 1 clearly and specifically states:

> "wherein a *lower level* of phosphorylated kinase substrate produced in the presence of said test compound *compared to* the level produced in the absence of said test compound indicates that said test compound is an inhibitor of mitosis." (emphasis added).

Applicants submit that there is nothing improper or indefinite with the grammar of the above-quoted section of Claim 1. A person skilled in the art would understand that the context of the above-quoted portion of Claim 1 makes clear that the term "a lower level" with respect to the phosphorylated kinase substrate produced in the presence of the test compound is "lower" when "compared to" the level produced in the absence of the test compound and that only when there is such a comparatively (or relatively) lower level of phosphorylated substrate produced is the test compound identified as an inhibitor of mitosis. Thus, "a lower level" is indeed a relative term, and the relationship between levels of phosphorylated kinase substrate are clearly and definitively recited within Claim 1.

If the grammar of Claim 1 is not in issue, Applicants submit that the Examiner is trying to read into Applicants' claimed invention a requirement for a quantitative step that simply is not required to practice the invention, i.e., there is no requirement that a compound exceed a quantitative threshold of *potency* in order to be identified as an inhibitor of mitosis using Applicants' method. The skilled practitioner is fully capable of determining whether the level of Nercc1-mediated phosphorylation is relatively lower (or not) in the presence than in the absence of a compound as clearly stated in Claim 1. It is then up to the skilled practitioner to decide whether to develop further a compound so identified as an inhibitor of mitosis according to Applicants' claimed method. As there is no requirement under any statute that Applicants' claimed method identify only potent compounds that exhibit inhibitory properties, the use of a relative lowering of activity in the presence and absence of a test compound to practice the invention is understandable to a person skilled in the art in accordance with 35 U.S.C. 112, second

paragraph. Separating strong inhibitors from weak inhibitors after the method of the invention is practiced may be a worthwhile pursuit, but it is not a critical step in the present invention, which is effective to identify the totality of inhibitors, both weak and strong. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejections.

In section 9 of the Office Action, the Examiner rejected Claims 1-3, 8, and 11-16 as indefinite under 35 U.S.C. 112, second paragraph, for recitation of "Nercc1" and "Nek7". The Examiner mentioned that insertion of the appropriate sequence identifiers would obviate this rejection. As indicated above, Applicants have amended Claim 1 to add the sequence identifier "(SEQ ID NO:2)" after the term "Nercc1 kinase protein" and have amended Claim 8 to add the sequence identifier "(SEQ ID NO:6)" after the term "Nek7". Accordingly, the amendments made in accordance with the Examiner's directions obviate the rejections.

In section 10 of the Office Action, the Examiner rejected Claims 2, 3, 8, and 11-16 as indefinite under 35 U.S.C. 112, second paragraph, for recitation of the term "initially" in Claims 2 and 3. Applicants submit that the skilled practitioner who reads the specification would understand that the term "initially" in Claims 2 and 3 is used to simply emphasize the situation wherein the Nercc1 kinase protein is not an auto-activated Nercc1 kinase protein. See, e.g., page 6, lines 16-19, of the specification. Applicants further submit that a person skilled in the art is not prevented from practicing the invention with the term "initially" recited in Claims 2 and 3, and retention of the term has no affect on the metes and bounds of the claims. Nevertheless, deletion of the term also does not adversely affect the clarity and definiteness of the claim language or the ability of persons skilled in the art to practice the invention. Accordingly, as indicated above in the Listing of the Claims, Applicants have deleted the term from Claims 2 and 3 as superfluous. As noted by the Examiner, the deletion of the term obviates the rejections.

Rejections Under 35 U.S.C. 112, first paragraph

In section 11 on pages 7-12 of the Office Action, the Examiner rejected Claims 1-3, 8, and 11-16 under 35 U.S.C. 112, first paragraph, as lacking an enabling disclosure. In particular, the Examiner was of the view that the specification fails to describe a nexus between Nercc1 kinase-mediated phosphorylation of Nek7 and mitosis, between Nercc1 kinase activity and cancer, and between Nercc1 kinase activity and eukaryotic microbes. Applicants respectfully traverse. Even though Applicants believe that the relationships (nexuses) mentioned by the Examiner are clearly evident to persons skilled in this art who have read Applicants' specification, as explained below, none of them is actually necessary for a person skilled in the art to understand and practice the claimed invention.

Applicants have discovered that the Nercc1 kinase is a critical serine/threonine kinase in a cascade of kinases that regulates eukaryotic cell entry into and maintenance in mitosis. See, e.g., page 4, lines 16-27; page 23, lines 19-21; Examples 11-13, pages 55-69; of the specification. The discovery that

Nercc1 kinase plays a role in mitosis *is* the critical and only nexus that was necessary for Applicants to invent their claimed method wherein a compound that inhibits Nercc1 kinase activity in an *in vitro* assay is identified as an inhibitor of mitosis. Moreover, persons skilled in the art who have the benefit of having read the specification understand this role and further that a Nercc1 kinase protein is a serine/threonine kinase that has one kinase catalytic domain (see, e.g., Figure 1) and only one kinase activity, i.e., the enzymatic transfer of a phosphate group from a nucleoside triphosphate to a phosphate acceptor polypeptide molecule (kinase substrate). See, e.g., page 6, lines 14-30 and page 27, lines 16-22, of the specification. In addition, the phosphorylation of any polypeptide by a serine/threonine kinase is readily detected by methods well known in the art for assaying kinase reactions. Such methods include those mentioned in the specification such as the use of antibodies in immunodetection methods, e.g., immunnoprecipitation and/or immunoblotting methods, to detect the phosphorylated form of a kinase substrate (see, e.g., Example 7, page 48, line 24 - page 49, line 6, and Figure 4, which demonstrate use of a phospho-specific polyclonal antibody to phosphorylated Nek7), phosphoaminoacid analysis to detect phosphorylated amino acid residues, such as serine or threonine of a phosphorylated kinase substrate (see, e.g., page 46, lines 23 - 25, of the specification), and direct radiodetection of a radiolabeled kinase substrate molecule that has accepted a radiolabeled phosphate from a radiolabeled nucleoside triphosphate donor molecule (see, e.g., page 7, line 23 - page 8, line 5 and page 46, lines 25 - 27, of the specification). See, also, the classic description of versatile protein kinase assays by Roskoski, Jr., R., "Assays of Protein Kinase," *In* Methods In Enzymology, Vol. 99, Hormone Action, Part F, Protein Kinases, (Corbin and Hardman, eds.) (Academic Press, New York, 1983) pages 3-6; attached at Tab A.

The Nercc1 kinase is a serine/threonine kinase. See, e.g., page 46, lines 23-25 in Example 6, of the specification. A variety of polypeptides are known to persons skilled in the art that may be used as substrates in *in vitro* assays for such kinases. See, e.g., page 6, lines 16-30, of the specification. Furthermore, Applicants' specification provides results that illustrate that an *in vitro* assay for Nercc1 kinase activity may not only employ a physiological substrate, such as Nek6 or Nek7 (see, e.g., page 48, line 26-page 49, line 6 in Example 7 and page 66, lines 15-17 in Example 12, of the specification), but also such well known *in vitro* kinase substrates as histones (e.g., histone H3), myelin basic protein (MBP), and casein. See, e.g., page 46, lines 21-25 in Example 6 and page 66, lines 20-24 in Example 12, of the specification.

The metabolic "function" or "role" of a phosphorylated reaction product of an *in vitro* Nercc1 kinase assay is irrelevant to the practice of the claimed method, because the method is based on detecting whether or not a compound can inhibit Nercc1 mediated-phosphate transfer *in vitro* to an acceptor molecule, not whether the resulting phosphorylated reaction product, itself, also plays a role in mitosis. The function, if any, of the phosphorylated substrate is irrelevant to Applicants' invention, since the object

is accomplished by the fact of phosphorylation (not whether the phosphorylation or inhibition thereof has a medical use). Applicants' claimed method employs an *in vitro* assay to detect inhibition of the Nercc1 kinase activity, which Applicants have shown plays a critical role in the regulation of mitotic progression. Accordingly, persons skilled in the art who read Applicants' specification would understand that there is no reason to prove that there is a nexus between the phosphorylated kinase substrate of the assay and mitosis (even if one exists as in the case of Nek6 or Nek7) in order to understand and practice Applicants' claimed invention. Description so as to enable practice of the invention is the requirement of 35 U.S.C. 112, first paragraph.

At page 9 of the Office Action, the Examiner stated:

> "Thus, in view of the above, the claims read on identifying compounds for identifying inhibitors of mitosis that will treat cancer and/or eukaryotic microbial infections."

The above-quoted excerpt from the Office Action indicates that the Examiner is attributing an efficacy to products of the method of the invention that simply is not stated in any of Claims 1-3, 8, and 11-16. Applicants have discovered and claim a reliable way of testing compounds for the ability to inhibit mitosis based on their ability to inhibit *in vitro* the kinase activity of a Nercc1 kinase protein. Nercc1 kinase is shown by Applicants to play a critical role in a cascade of kinases that signals eukaryotic cells to enter and/or continue in mitosis. Some inhibitors of mitosis may indeed be candidates for treating cancer or eukaryotic microbial infection, as both types of diseases involve undesired mitotic progression, but such anti-cancer or anti-microbial activities are not required elements of the invention and properly are not required elements in any of the claims under examination. Applicants note in passing that compounds that inhibit mitosis have been and continue to be a well known source of potential anti-cancer agents. See, e.g., the review by Jackson et al., *Nature Reviews Cancer, 7*: 107-117 (2007) attached at Tab B. Perhaps the best known example of an inhibitor of mitosis that has been developed as an anti-cancer agent is paclitaxel (Taxol®, Bristol-Myers Squibb, Princeton, New Jersey). See, paclitaxel patient information sheets attached at Tab C. Nevertheless, such potential utilities are not part of Applicants' claimed invention for identifying an inhibitor of mitosis. Indeed, Applicants' disclosure specifically teaches that compounds identified as inhibitors of mitosis may be further investigated for one or more therapeutic activities:

> "Compounds initially identified as inhibitors of mitosis (i.e., anti-mitotic compounds) according to the method described above may be further characterized for the ability to inhibit or halt mitosis in proliferating (actively dividing) cells, either *in vitro* or *in vivo*. The cells employed in this further characterization step may be any of a variety of proliferating cells, including but not limited to, non-cancerous cells, cancer cells, or cells of a eukaryotic pathogen of interest. Preferably, the proliferating

> cells are cancer cells or cells of a eukaryotic pathogen of interest. (page 29, lines 11-17, of the specification; underlining added for emphasis).

Accordingly, Applicants clearly recognized that a compound that is identified as an inhibitor of mitosis based on its ability to inhibit Nercc1 kinase activity in an *in vitro* assay may also provide one or more therapeutic activities. However, the effectiveness of any compound identified as an inhibitor of mitosis according to Applicants' claimed method in treating cancer or a eukaryotic microbial infection is simply not an object or required element of the claimed invention. Confirmation of such therapeutic benefits requires carrying out further steps to characterize the inhibitor compound (see, e.g., withdrawn Claims 17-19), but such additional steps are not a critical feature of the claimed invention.

In view of the above comments, Applicants respectfully submit that persons skilled in the art who have the benefit of having read the specification would understand that the claimed invention is properly based on the discovery of that Nercc1 kinase activity plays a role in regulating cell entry into and/or maintenance of mitosis and that no other biochemical relationship needs to be known or shown to practice the claimed invention. Accordingly, the specification fully supports Applicants' claims and no undue experimentation is required to practice the claimed invention under 35 U.S.C. 112, first paragraph. Applicants therefore respectfully request that the Examiner reconsider and withdraw the rejections.

At section 12 in the Office Action, the Examiner rejected Claims 1-3, 8, and 11-16 under 35 U.S.C. 112, first paragraph, as overly broad with respect to recitation of a Nercc1 kinase protein and a Nek7 kinase substrate. Applicants traverse the rejections for the reasons given below.

As explained above, Applicants discovered that the Nercc1 kinase protein plays a critical role in a cascade of kinases that regulates eukaryotic cell entry into and/or maintenance in mitosis. The activity of the Nercc1 kinase is the enzymatic transfer of a phosphate from a nucleoside triphosphate donor molecule to a kinase substrate that accepts the phosphate. The Nercc1 kinase activity is easily assayed *in vitro* using a Nercc1 kinase protein and a kinase substrate that is susceptible to phosphorylation by the Nercc1 kinase protein. A kinase substrate for use in the *in vitro* Nercc1 kinase assay may be a physiological substrate, such as Nek6 or Nek7, or any of a variety of other polypeptides that are routinely employed as phosphate acceptors for *in vitro* serine/threonine kinase assays. In addition, as noted above, a variety of methods are known in the art by which a polypeptide that has been phosphorylated in an *in vitro* kinase reaction assay may be detected. Accordingly, the elements and techniques that a person skilled in the art can use to practice the invention are fully supported by the specification. Nevertheless, to advance prosecution, Applicants have amended the claims by inserting sequence identifiers that, respectively, specify that the Nercc1 kinase protein has the amino acid sequence of SEQ ID NO:2 (Claim 1) and that the Nek7 kinase substrate has the amino acid sequence of SEQ ID NO:6 (Claim 8). In view of the amendments to the claims, Applicants respectfully request that the Examiner withdraw the rejections.

In section 13 of the Office Action, the Examiner rejected Claims 1-3, 8, and 11-16 under 35 U.S.C. 112, first paragraph, as lacking an adequate written description. Applicants respectfully traverse the rejections for the reasons given below.

As shown in the Listing of the Claims and explained above, Applicants have amended the claims to specify that the Nercc1 kinase protein has the amino acid sequence of SEQ ID NO:2 and that the Nek7 kinase substrate has the amino acid sequence of SEQ ID NO:6. In addition, Applicants have also incorporated into Claim 1 further descriptive text from the specification that the kinase substrate is a polypeptide that comprises a domain that is susceptible to phosophorylation by the Nercc1 kinase protein. Moreover, a variety of kinase substrates may be used in the claimed invention. See, e.g., page 6, lines 14-30, of the specification. Applicants respectfully submit that the aforementioned amendments to the claims clearly reflect an adequate written description of the claimed invention provided by the specification and obviate the Examiner's rejections. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections to the claims.

Examiner's Determination of Closest Prior Art

Applicants acknowledge with appreciation the Examiner's determination that Holland et al., *J. Biol. Chem., 277*: 16229-16240 (2002) constitutes the closest prior art and describes a Nek8 protein that may be associated with cell cycle independent microtubule dynamics. Applicants specifically note that no art has been relied on to reject the claims.

In view of all of the above comments and the amendments to the claims, Applicants respectfully submit that the Examiner's rejections have been overcome, have been obviated, or have been rendered moot. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejections of Claims 1-3, 8, and 11-16, as amended herein, and pass this application to issue.

Respectfully submitted,

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[1] Assays of Protein Kinase

By ROBERT ROSKOSKI, JR.

Assay Method

$$\text{ATP} + \text{protein} \rightarrow \text{phosphoprotein} + \text{ADP}$$

Principle. Protein kinases catalyze the transfer of the γ-phosphoryl group of ATP to an acceptor protein substrate. Their activity is conveniently measured by using [γ-^{32}P]ATP and an appropriate acceptor substrate. Unreacted ATP and its metabolites are then resolved from the radioactive protein substrate by a variety of techniques. One widely used procedure to achieve this involves protein precipitation in cellulose strips by trichloroacetic acid followed by extensive washing.[1] We developed an alternative procedure for resolving phosphohistone from ATP and its metabolites based upon adsorption of phosphohistone onto phosphocellulose strips.[2] ATP and its metabolites, on the other hand, fail to bind to phosphocellulose. Using the synthetic Ser-peptide (Leu Arg Arg Ala Ser Leu Gly) as substrate, Glass and co-workers used 30% acetic acid to effect binding of the phosphopeptide to phosphocellulose discs.[3] We have subsequently developed a general method for studying both peptide and protein phosphorylation using phosphoric acid to convert the acceptor peptide or protein substrates into positively charged forms which bind to the phosphocellulose paper.[4] The phosphoric acid, moreover, more effectively displaces ATP (and its metabolites) from the phosphocellulose strips than does water or 30% acetic acid.

In addition to measuring the activity of cyclic nucleotide-dependent protein kinases, the phosphocellulose method can be used for phosphorylase kinase, myosin light chain kinase, and other protein kinase reactions. To document specificity, phosphate incorporation should be dependent upon added peptide or protein substrate.

Reagents

Morpholinopropanesulfonic acid (MOPS), pH 7.0, 500 m*M*; 100 m*M* $MgCl_2$, bovine serum albumin, 2.5 mg/ml

[1] J. D. Corbin and E. M. Reimann, this series, Vol. 38C, p. 287.
[2] J. J. Witt and R. Roskoski, Jr., *Anal. Biochem.* **66,** 253 (1975).
[3] D. B. Glass, R. A. Masaracchia, J. R. Feramisco, and B. E. Kemp, *Anal. Biochem.* **87,** 566 (1978).
[4] P. F. Cook, M. E. Neville, K. E. Vrana, F. T. Hartl, and R. Roskoski, Jr., *Biochemistry* **21,** 5794 (1982).

ISBN 0-12-181999-X

Histone (e.g., Sigma histone type II A mixture), 10 mg/ml, or Ser-peptide (e.g., Peninsula Laboratories, Leu Arg Arg Ala Ser Leu Gly), 1.0 m*M* (or other acceptor protein substrate)
[γ-^{32}P]ATP, 1.0 m*M*, 100 cpm/pmol
Cyclic AMP, 100 μM
Protein kinase
Phosphoric acid (5 ml/liter of 85% phosphoric acid, 75 m*M*)

Procedure. The protein kinase reaction mixture contains 50 m*M* MOPS (pH 7.0), 10 m*M* $MgCl_2$, 0.25 mg/ml bovine serum albumin, 1.0 mg/ml histone or 100 μM Ser-peptide (or other acceptor protein), 100 μM ATP, and 10 μM cAMP (if desired), H_2O and enzyme to give a final volume of 50 μl. The reaction is initiated by enzyme addition. Following incubation (2–10 min as desired), portions (25 μl) are withdrawn, spotted onto 1 × 2-cm phosphocellulose strips (e.g., Whatman P81) and immersed in 75 m*M* phosphoric acid (10 ml per sample) to terminate the reaction. Samples lacking enzyme (blanks) are added last since the desorption of labeled ATP is time dependent.

The strips are swirled gently for 2 min, the phosphoric acid is decanted, and the phosphocellulose strips are washed twice more (2 min each) in phosphoric acid with gentle agitation. The stirring may be performed with a stirring rod or with a magnetic stirrer. The agitation must be carefully monitored and gentle because the phosphocellulose strips are fragile. After drying (optional) in air, with a hair dryer, or in an oven (100°, 5 min), the radioactivity is measured by liquid scintillation spectrometry with Budget-Solve or other appropriate scintillant or by measuring Cerenkov radiation (in the absence of scintillant). After application of 250,000 cpm, blanks are less than 250 cpm. With a fourth 2-min wash, blanks of less than 100 cpm are obtained.

Spectrophotometric Assay

$$\text{ATP + peptide} \rightarrow \text{phosphopeptide + ADP}$$
$$\text{ADP + phosphoenolpyruvate} \rightarrow \text{ATP + pyruvate}$$
$$\text{pyruvate + DPNH} \rightarrow \text{lactate + DPN}^+$$

Principle. With this technique, the formation of ADP in the protein kinase reaction is coupled to the pyruvate kinase reaction to produce pyruvate which is, in turn, coupled to the lactate dehydrogenase reaction with the concomitant oxidation of DPNH to DPN^+.[4] The decrease in absorbance at 340 nm is used to determine the reaction rate using an extinction coefficient of 6220 M^{-1} cm^{-1}. The optimum concentrations of

lactate dehydrogenase and pyruvate kinase were determined according to Cleland[5] and validated by varying the protein kinase concentration.

Reagents

0.5 *M* MOPS (pH 7.0), 0.5 *M* KCl, 50 m*M* $MgCl_2$
10 m*M* ATP
10 m*M* PEP
1 m*M* Ser-peptide
3000 units/ml pig heart lactate dehydrogenase
1400 units/ml rabbit muscle pyruvate kinase
Cyclic AMP-dependent protein kinase catalytic subunit (0.017 units = 890 ng)
100 m*M* DPNH (stored at 0° for less than 10 days)

Procedure. The final 1-ml incubation mixture contains the following components: 100 m*M* MOPS, 15 U lactate dehydrogenase, 7 U pyruvate kinase, 100 m*M* KCl, 1 m*M* phosphoenolpyruvate, 10 m*M* $MgCl_2$, 1 m*M* ATP, 100 μM Ser-peptide, 200 μM DPNH. Contaminant ADP is converted to ATP instantaneously. After recording the minor ATPase activity of the components, the reaction is initiated by addition of enzyme (usually 2 μl). The background rate (ATPase activity) is subtracted from the rate obtained in the presence of C subunit. The rates are monitored at 340 nm with a signal output of 0.05 to 0.1 full-scale absorbance. A plot of velocity against concentration of C subunit is linear up to at least 0.08 units (4 μg/ml).

Discussion

The radioisotopic method for cAMP-dependent protein kinase can be used with tissue homogenates and purified enzyme. Because crude homogenates contain ATPase activity and the heat-stable protein kinase inhibitor,[6] activities obtained following DEAE-cellulose chromatography are greater than that of the initial homogenate for brain, heart, skeletal muscle, and liver.

The method is generally applicable for all acceptor proteins except those acidic proteins which are not positively charged at pH 1.8. Small basic peptides such as Ser-peptide can also be employed. The initial peptide substrate should have a charge of +3 at pH 1.8 for the method to work. *N*-Acetyl Ser-peptide, for example, is incompletely recovered on the phosphocellulose under these conditions. The phosphoric acid procedure is preferable to 30% acetic acid for several reasons. First, the non-

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specific absorption is less. This may be due to the displacement of ATP and its metabolites by the higher concentration of phosphate. Furthermore, it is more rapid, economical and the strips are easier to handle in dilute phosphoric acid than in 30% acetic acid.

The phosphoric acid procedure can be used to monitor autophosphorylation of the type II R subunit and to measure incorporation of labeled substances into the C subunit. The same stoichiometries are obtained with this procedure as with trichloroacetic acid precipitation. The capacity of the paper is much greater than the amount of acceptor substrates commonly employed (up to 10 mg/ml protein or 250 μM peptide) per 25-μl portion. If very high concentrations are used, then the capacity of the paper should be checked to ensure that binding is complete.

The spectrophotometric assay is valuable in enzyme kinetic studies because it is continuous. That the experimental conditions yield initial velocities is apparent immediately and not after a series of assays have been performed as with the radioisotopic method. Any nucleoside which participates in the pyruvate kinase couple can be used and synthesis of radiolabeled nucleotide is not required. Phosphorylation of synthetic peptides which fail to bind completely to the phosphocellulose (*N*-acetyl Ser-peptide) can be measured with the spectrophotometric assay. The assay can be used to measure activity in preparative DEAE-cellulose chromatographic fractions. Most of the ATPase activity observed is Ser-peptide and cAMP-dependent. The radioisotopic assay, however, is much more rapid for processing the large numbers of fractions associated with enzyme purification.

The cAMP-dependent protein kinase exhibits a broad pH optimum. Buffers other than MOPS can be effectively employed. The enzyme is sensitive to conditions of ionic strength and particularly Mg^{2+} concentration. Increasing the free Mg^{2+} from 1 to 10 mM decreases the K_m fivefold (50 to 10 μM) and also decreases the V_{max} fivefold.[4] When using low concentrations of high specific activity ATP, the 10 mM $MgCl_2$ decreases the K_m to give higher incorporation, but decreases the V_{max}.

The synthetic acceptor peptide obtained from commercial sources (Sigma Chemical Co., Boehringer-Manheim, and Peninsula Laboratories) contains 25–50% nonpeptide material by weight. This was determined both spectrophotometrically and by the radioisotopic procedure using limiting acceptor peptide. In the radioisotopic procedure, a 20-fold excess of [γ-^{32}P]ATP can be used to make this determination.

Acknowledgments

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[2] Measurement of Chemical Phosphate in Proteins

By JANICE E. BUSS and JAMES T. STULL

Analytical procedures for measuring the phosphate content in proteins have suffered in general from a lack of sensitivity. This problem has required preparation of large amounts of a purified protein for phosphate analysis, a task which cannot easily be accomplished for many phosphoproteins. Such preparation is particularly difficult in cases in which the protein must be purified from tissue biopsy samples obtained for investigations of protein phosphorylation *in vivo*. The sensitivity of the procedure described below is 30 times greater than the standard Fiske–SubbaRow procedure for measuring inorganic phosphate and measures as low as 0.2 nmol phosphate. This procedure incorporates two methods. First, the purified protein sample is ashed to convert protein-bound phosphate to inorganic phosphate. Second, the inorganic phosphate is measured after complexation of phosphomolybdate with the triphenylmethane dye, malachite green.

Preparation of Proteins

The direct chemical measurement of phosphate covalently bound to a specific protein can be attempted only on proteins that are free of other phosphate-containing compounds. Therefore, it is essential that during the early stages of investigation both the nature of the phosphate bond to be characterized and the extent of nonprotein phosphate contamination be determined. This applies both to proteins isolated in such abundance that the fortunate investigator may monitor purification by simple protein assays and to proteins that require identification or assay with radioactive phosphate probes.

Noncovalent association of phosphate-containing compounds with proteins may be of three general types: low-molecular-weight cofactors or regulatory groups such as nucleotides, pyridoxal phosphate, or phosphosugars, nucleic acids, or phospholipids.[1] The fact that the association is noncovalent does not prevent the compound from interacting with the protein with an affinity sufficient to confound assays of protein-bound phosphate. The affinity of 3′ : 5′-cyclic adenosine monophosphate (cyclic AMP) for the type I regulatory subunit of cyclic AMP-dependent protein kinase is sufficiently high that dialysis against 4 M urea is necessary for

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Targeted anti-mitotic therapies: can we improve on tubulin agents?

Jeffrey R. Jackson, Denis R. Patrick*, Mohammed M. Dar* and Pearl S. Huang‡*

Abstract | The advent of molecularly targeted drug discovery has facilitated the identification of a new generation of anti-mitotic therapies that target proteins with specific functions in mitosis. The exquisite selectivity for mitosis and the distinct ways in which these new agents interfere with mitosis provides the potential to not only overcome certain limitations of current tubulin-targeted anti-mitotic drugs, but to expand the scope of clinical efficacy that those drugs have established. The development of these new anti-mitotic drugs as targeted therapies faces significant challenges; nevertheless, these potential therapies also serve as unique tools to dissect the molecular mechanisms of the mitotic-checkpoint response.

Pharmacodynamic markers
A molecular marker of drug response that can be measured in patients receiving the drug. The marker should be a direct measure of modulation of the drug target and be able to show quantitative changes in response to dose.

Inducing aberrant mitosis in tumour cells leads to mitotic arrest, the consequence of which can be, but is not always, cell death. Given the proven success of therapies that change microtubule dynamics such as the vinca alkaloids and taxanes in the clinical treatment of cancer (TABLE 1), it is reasonable to consider non-structural components of mitosis as potential drug targets for therapy. The recent explication of mitosis into discrete morphological stages mediated by defined biochemical effectors has defined additional functions for the mitotic kinesins, Aurora kinases and polo-like kinases (PLKs), all of which are druggable target classes[1] (FIG. 1, TABLE 2). The dynamic assembly of the mitotic spindle is well characterized and has been reviewed elsewhere[2]. Although the rationale for targeting Aurora A, Aurora B[3] and PLK1 (REF. 4) for cancer treatment are relatively well described, the mitotic kinesin, kinesin spindle protein (KSP; also known as EG5)[5], which is required for progression from prophase to prometaphase, and centromeric protein E (CENPE), which functions during transition from prometaphase to metaphase[6,7] and is a component of the mitotic checkpoint[8], have only recently emerged as cancer treatment targets.

That these targets are only expressed in dividing cells is attractive, as non-dividing differentiated cells should not be affected by target inhibition, therefore potentially enabling an improved therapeutic index relative to the existing anti-mitotic therapies that target tubulin. Nevertheless, some of the anti-tumour effects of the tubulin drugs might be attributed to interphase interactions with the tubulin cytoskeleton. The newer targets might not afford the opportunity to alter both the mitotic spindle and the cytoskeleton, although the potential for undiscovered roles outside mitosis remains. In further support of new mitotic targets, the explication of these molecular targets and pathways during mitosis enables target inhibition to be associated with tumour growth inhibition, and suggests the possibility of using pharmacodynamic markers to determine biologically effective dosing during drug development. The ability to dose to an effective level rather than to a maximally tolerated dose might also enable improved therapeutic indicies with these targeted anti-mitotic agents.

Although there are many positive aspects to targeting the mitotic kinases and kinesins, the molecular pathways through which tumour cells undergo cell death in response to mitotic arrest are not well defined. Apoptosis characterized by the activation of caspase 3 (an important downstream effector) has been observed in studies with many anti-mitotic agents[9–11]. However, an alternative mechanism of cell death termed mitotic catastrophe has also been described. Mitotic catastrophe does not have a strict definition, but shares many of the morphological and biochemical aspects of apoptosis[12]. It has been described as cell death that occurs from metaphase of mitosis in response to agents that cause DNA or mitotic-spindle damage. In this form it can be independent of caspase 3 and involve the activation of caspase 2. In addition, the role of the mitotic checkpoint (FIG. 2) as an effector of cell death in response to mitotic inhibitors is controversial. An intact mitotic checkpoint has been suggested as a requirement for response to a KSP inhibitor[9]; however, an alternative theory of the role of checkpoint signalling during mitosis suggests that the

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At a glance

- Anti-mitotic therapies that target tubulin are effective and widely used in treating cancer, but they have limitations related to the role of tubulin in the cytoskeleton of normal cells.
- New compounds that inhibit new targets with specific functions in mitosis have now been identified, and show promising anti-tumour activity in preclinical model systems.
- Early clinical studies have begun to show the pharmacodynamic activities of these new compounds in cancer patients.
- These new mitotic inhibitors are very effective at preventing the proliferation of most tumour cells *in vitro*, but the subsequent cellular response to cell-cycle arrest is quite varied and includes apoptosis, mitotic catastrophe, mitotic slippage, senescence and reversible mitotic arrest depending on what cell line and/or inhibitor is studied.
- At present, the genetic or biochemical factors that define how a particular tumour cell will respond to mitotic injuries are poorly understood, but will be very important in helping to identify which patients will be the best candidates for treatment with these new agents.

loss of checkpoints might permit cell death in response to mitotic damage or even be lethal alone[13–15]. Aurora B inhibitors seem to promote the latter mechanism[16]. It has become increasingly clear that the inhibition of different target proteins that function during mitosis can kill cells by distinct mechanisms and, furthermore, these mechanisms can be influenced by various genetic alterations that are often found in cancer. Nevertheless, it is difficult at present to use molecular means of preselecting which tumours are likely to respond to a particular targeted inhibitor of mitosis because our understanding of these downstream mechanisms is incomplete.

In this Review we address what is known about the function of the mitotic kinesins and the mitotic kinases, and whether this knowledge can be used to identify reliable biomarkers that will predict the likelihood of tumour sensitivity or resistance to inhibitors of mitosis.

Mitotic kinesins

Although most compounds discovered to date that directly perturb the function of the mitotic spindle have turned out to be drugs that bind tubulin, there are many additional proteins that have crucial roles in the mechanics of mitosis and in progression through the mitotic cell-cycle checkpoint. Although most have not proven to be amenable to pharmacological modulation, the mitotic kinesins, a subgroup of kinesin motor proteins that function exclusively in mitosis, have recently emerged as a druggable target class[1]. Different mitotic kinesins have crucial roles in unique aspects of spindle assembly and function, including spindle-pole organization, chromosome alignment and segregation, and the regulation of microtubule dynamics (FIG. 1).

KSP. KSP is a kinesin motor protein required to establish mitotic-spindle bipolarity[5]. So far no role for KSP outside mitosis has been found. Orthologues of KSP have been identified in various organisms, including tobacco plants, slime molds, fission yeast, budding yeast, fruit flies, frogs and humans[17]. In all model organisms and cultured human cells examined to date, KSP has a similar role in driving centrosome separation to establish the bipolar spindle. The expression profiles of *KSP* mRNA in normal human tissues are consistent with preferential expression in proliferating cells compared with non-proliferating cells, and overexpression in tumour tissue compared with normal adjacent tissue[18].

Table 1 | **Anti-mitotic drugs in clinical use**

Agent	Microtubule mechanism of action	Approved indications (cancer types)	Investigational uses (cancer types)	Myelosuppression*	Gastrointestinal‡	Neuropathy	Alopecia	Other
Docetaxel	Stabilizer	Adjuvant and advanced breast; non-small-cell lung; androgen-independent prostate	Gastric; pancreatic; head and neck; ovarian; soft tissue sarcoma; melanoma	++	++	++	+++	Fluid retention ++
Paclitaxel	Stabilizer	Adjuvant and advanced breast; ovarian; non-small-cell lung; AIDS-related Kaposi sarcoma	Bladder; cervical; prostate; head and neck	++	++	++	+++	
Vinblastine	De-stabilizer	Hodgkin lymphoma; non-Hodgkin lymphoma, testicular; Kaposi sarcoma	Bladder	+++	+	+	+	SIADH§ +
Vincristine	De-stabilizer	Acute leukaemias; Hodgkin and non-Hodgkin lymphoma; neuroblastoma; rhabdomyosarcoma; Wilms tumour	Myeloma; small-cell lung; brain	–	–	+++	++	
Vinorelbine	De-stabilizer	Non-small-cell lung	Advanced breast, ovarian, Hodgkin and non-Hodgkin lymphoma	+++	++	+	+	

*Includes any of the following: neutropaenia, leukopaenia, anaemia, thrombocytopaenia. ‡Includes any of the following: mucositis; nausea/vomiting; diarrhoea; increased levels of hepatic transaminases; increased levels of bilirubin. §Syndrome of inappropriate antidiuretic hormone secretion.

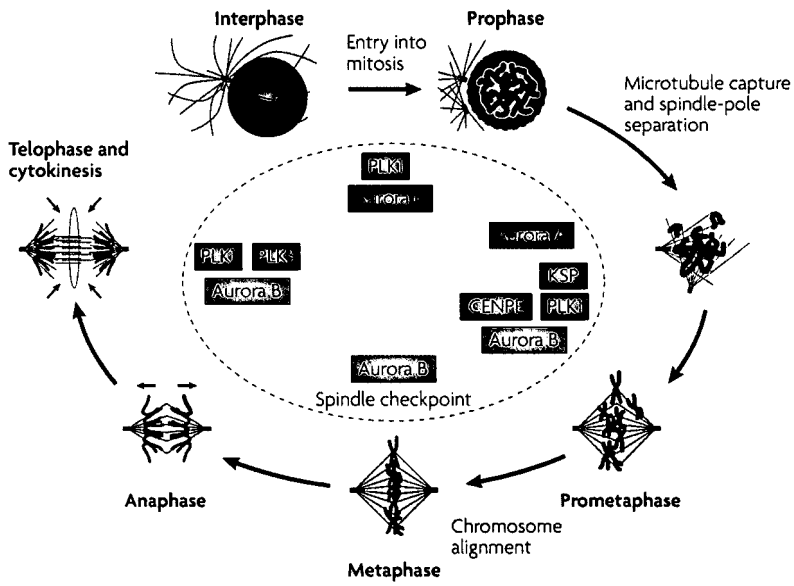

Figure 1 | **The phases of mitosis.** The progression of mitosis through the canonical morphological stages is shown. Specific druggable protein targets that function during mitosis are highlighted. Kinesin spindle protein (KSP) is required to establish mitotic spindle bipolarity through driving centrosome separation. Centromeric protein E (CENPE) is required for accurate chromosome congression at metaphase during mitosis. Aurora A is crucial for centrosome maturation and separation during early prophase. Aurora B is a member of the chromosomal passenger complex (CPC) and is involved in histone H3 phosphorylation, chromosomal condensation, chromosomal alignment on the metaphase plate, bi-polar centromere-microtubule attachments, spindle checkpoint and cytokinesis. During mitosis, Polo-like kinase 1 (PLK1) is involved in centrosome maturation and formation of the mitotic spindle. PLK1 is also required for exit from mitosis and the separation of sister chromatids during anaphase. PLK1 might also have a role in cytokinesis through the phosphorylation of the kinesin-like motor protein MKLP1.

Monopolar spindle
A mitotic spindle in which the centrosomes are unseparated. A monopolar spindle is incapable of separating the sister chromatids because the spindle poles are not oriented on opposite sides of the chromosomes.

Spindle tension
In a properly functioning mitotic spindle, the microtubules that connect the chromosomes to the centrosomes are under tension. This tension, which creates a pulling force toward the centrosome, can be created by a poleward flux of tubulin within the microtubule, as well as kinesin and dynein motor proteins.

Bi-orientation
This describes chromosomes that have microtubule attatchments to both spindle poles.

The first inhibitor of KSP to be reported was monastrol, which was identified in a cell-based screen looking for compounds that caused mitotic arrest without direct effects on microtubule dynamics[19]. Subsequently, several more potent KSP inhibitors have been reported in the scientific literature[20–24], and dozens have been reported in the patent literature[25]. At least three have entered into clinical trials (TABLE 3). The inhibitors reported so far have the unique property of being highly selective for KSP. This is because they bind to an allosteric site adjacent to loop 5 (REF. 26), and this site is not present in other kinesins, including those that are highly structurally related. Because of this property, KSP inhibitors are excellent tools for understanding the function of KSP motor activity in both *in vitro* and *in vivo* disease models, as well as in a clinical setting.

The effect of KSP inhibitors on proliferating cells is to cause a mitotic arrest with a monopolar spindle. The centrosomes do not separate, so the microtubules are organized from a single site in the cells and the chromosomes orientate in a ring around this site. This phenotype is ubiquitous to all proliferating cells treated with KSP inhibitors, so these agents are very effective at inhibiting cell proliferation. There are no reported effects on non-proliferating cells using relevant concentrations of these compounds. Mitotic arrest induced by KSP inhibitors has been shown to result in apoptosis in some tumour cell lines[9,11]. A quinazolinone inhibitor of KSP (CK0106023) was tested in a SKOV3 human tumour xenograft model and showed inhibition of tumour growth comparable to paclitaxel[20]. Other KSP inhibitors such as SB-715992 (REFS 27,28) and MK-0731 (REF. 29) have shown anti-proliferative activity in tumour cell lines and significant efficacy in several murine tumour models.

CENPE. CENPE, a new mitotic kinesin target composed of 2,663 amino acids, is both an effector and sensor of mitotic events. The motor domain of CENPE is required for accurate chromosome congression at metaphase during mitosis. The crucial domains of CENPE include a motor domain with ATPase activity, a neck domain, a microtubule-binding domain and a cargo-binding domain for the kinetochore[7,30–32]. CENPE binds to and regulates the mitotic checkpoint kinase, BUBR1, at kinetochores[33,34] (FIG. 2). The simultaneous attachment of CENPE to microtubules and the kinetochore is required for the mitotic checkpoint to be passed, which enables progression to anaphase. Cells in which CENPE protein has been inhibited or deleted characteristically show spindle-pole fragmentation, loss of chromosome alignment during congression and reduced spindle tension[6,7,35,36]. Observations made using videomicroscopy and short interfering RNA (siRNA) to ablate CENPE protein expression show the accumulation of mono-oriented sister chromatids where only one kinetochore is attached to growing microtubles. These observations suggest that CENPE is responsible for guiding the unattached sister kinetochores into the proximity of distal microtubules, enabling the congression of chromosomes at the spindle equator before bi-orientation[37].

Curiously, the induction of cell death in response to the ablation of CENPE is not always observed. In regenerating liver cells from CENPE conditional knockout mice, cells are capable of several rounds of replication, despite incomplete chromosome alignment[38]. Therefore, the role of CENPE as a component of the mitotic checkpoint, rather than an essential driver of mitotic progression, makes it a unique target for mitosis, taking advantage of the potential differences between tumour cells and their normal dividing counterparts. Inhibitors of CENPE have been identified and are now in preclinical development (J.R.J. *et al.*, unpublished data).

Aurora and polo-like kinases

The Aurora kinases are a family of mitotic kinases (BOX 1) that are receiving significant attention in both the academic and pharmaceutical communities. These kinases have been the subject of recent reviews and will not be reviewed in depth here[3,39–41]. Studies using siRNA, microinjected antibodies and selective small-molecule inhibitors of Aurora A[42] lead to cells with severely defective spindle morphology[43,44] (D.R.P. *et al.*, unpublished data). Inhibition of the Aurora B kinase provides a unique mechanism of action for inhibiting mitosis that is distinct from the current anti-mitotic agents and from inhibitors of the mitotic kinesins. SiRNA and small-molecule inhibitors of Aurora B abrogate the

Table 2 | ***In vitro* activities of new mitotic-targeted agents**

Compound	Company	Enzymatic potency	Cellular potency	Comments
Aurora inhibitors				
MK-0457 (VX-680)	Vertex/Merck	Aurora A 0.7 nM; Aurora B 18.0 nM; Aurora C 4.6 nM	Typical anti-proliferation IC_{50}s of 10–100 nM	Pan Aurora inhibitor; also inhibits BCR-ABL wild type and T315I mutant at ~30 nM; FLT3 IC_{50} = 30 nM
AZD1152	Astra Zeneca	Aurora A 1369 nM; Aurora B 0.36 nM; Aurora C 17 nM	11 nM EC_{50} for cell death	Aurora B selective
PHA-680632	Nerviano Medical Sciences	Aurora A 27 nM; Aurora B 135 nM; Aurora C 120 nM	60–600 nM for anti-proliferation	FGFR1 390 nM
MLN8054	Millennium	Aurora A 4 nM; Aurora B 172 nM	Aurora A (pT388) 34 nM; Aurora B (pHH3) 5700 nM	Aurora A selective
CENPE inhibitors				
GSK-923295	GlaxoSmithKline/ Cytokinetics	No data		
KSP inhibitors				
Ispinesib (SB-715992)	Cytokinetics	KSP Ki = 0.6 nM; >40,000-fold selective for KSP versus other kinesins	<10 nM IC_{50} for tumour cell proliferation	
SB-743921	Cytokinetics	KSP Ki = 0.1 nM; >70,000-fold selective for KSP versus other kinesins	0.06–2 nM IC_{50} for tumour cell proliferation	
MK-0731	Merck	KSP IC_{50} = 2 nM; >20,000-fold selective for KSP versus other kinesins	3–5 nM IC_{50} for tumour cell proliferation	
ARRY-649	Array BioPharma	KSP IC_{50} = 0.7 nM	0.2 nM IC_{50} for tumour cell proliferation	Selectivity not reported
PLK inhibitors				
BI2536	Boehringer Ingelheim	PLK1 IC_{50} = 0.8 nM	4–25 nM IC_{50} for tumour cell proliferation	>10,000-fold selective for PLK1 versus 43 other kinases
ON 01910	Onconova	PLK1 IC_{50} = 9 nM	30–45 ng/ml IC_{50} for tumour cell proliferation	Inhibits several kinases in addition to PLKs. Not ATP competitive, peptide substrate competitive

CENPE, centromere protein E; EC_{50}, the concentration at which a drug has 50% of its maximal effect; FLT3, fms-related tyrosine kinase 3; IC_{50}, the concentration of a an inhibitor that is required for 50% inhibition of its target *in vitro*; KSP, kinesin spindle protein; PLK1, polo-like kinase 1.

mitotic-spindle checkpoint and cause premature mitotic exit without the completion of cytokinesis, leading to 4N DNA-containing cells that continue to progress through the cell cycle[16,45,46]. Continued Aurora B inhibition causes cells to undergo several rounds of the cell cycle without cytokinesis, which results in very large multiploid cells that eventually undergo apoptosis or occasionally senescence[47] (D.R.P. *et al.*, unpublished data). Several studies have shown that complete inactivation of the spindle checkpoint is lethal, whereas partial inactivation promotes tumorigenesis[13–15]. As Aurora B activity is required to induce the spindle checkpoint, Aurora B inhibitors have a dominant phenotype when combined with other anti-mitotic agents including Aurora A inhibitors. Dual Aurora B + A inhibitors or combinations of siRNA's targeted to both kinases result in large multiploid cells[48].

Aurora B inhibitors clearly have a cellular mechanism of action that is distinct from traditional anti-mitotic agents in that cells continue to cycle instead of arresting in mitosis. One potentially significant advantage to this mechanism is that Aurora B inhibitors could be combined with agents that require exposure during other phases of the cell cycle. Timing or the order of addition could be crucial for maximum efficacy. Presumably, exposure to an Aurora B inhibitor would have to precede any other agent that causes an arrest outside mitosis. Recently reported results support this hypothesis. Chronic myeloid leukaemia (CML) cells treated initally with an Aurora kinase inhibitor followed by idarubicin or cytosine arbinoside showed a greater loss in viability than cells that were treated with the two agents simultaneously[49]. Conversely, combining Aurora B inhibitors with other anti-mitotic agents might not be productive, as they typically require a significant mitotic arrest for efficacy.

Polo-like kinases (PLKs) are evolutionarily conserved serine or threonine kinases that have crucial roles in regulating cell-cycle processes in diverse organisms from yeast to mammalian cells[50] (BOX 2). PLK1 is the human homologue of *Drosophila* polo, and is involved in the entry to mitosis through the activation of cyclin-dependent kinase 1 (CDK1).

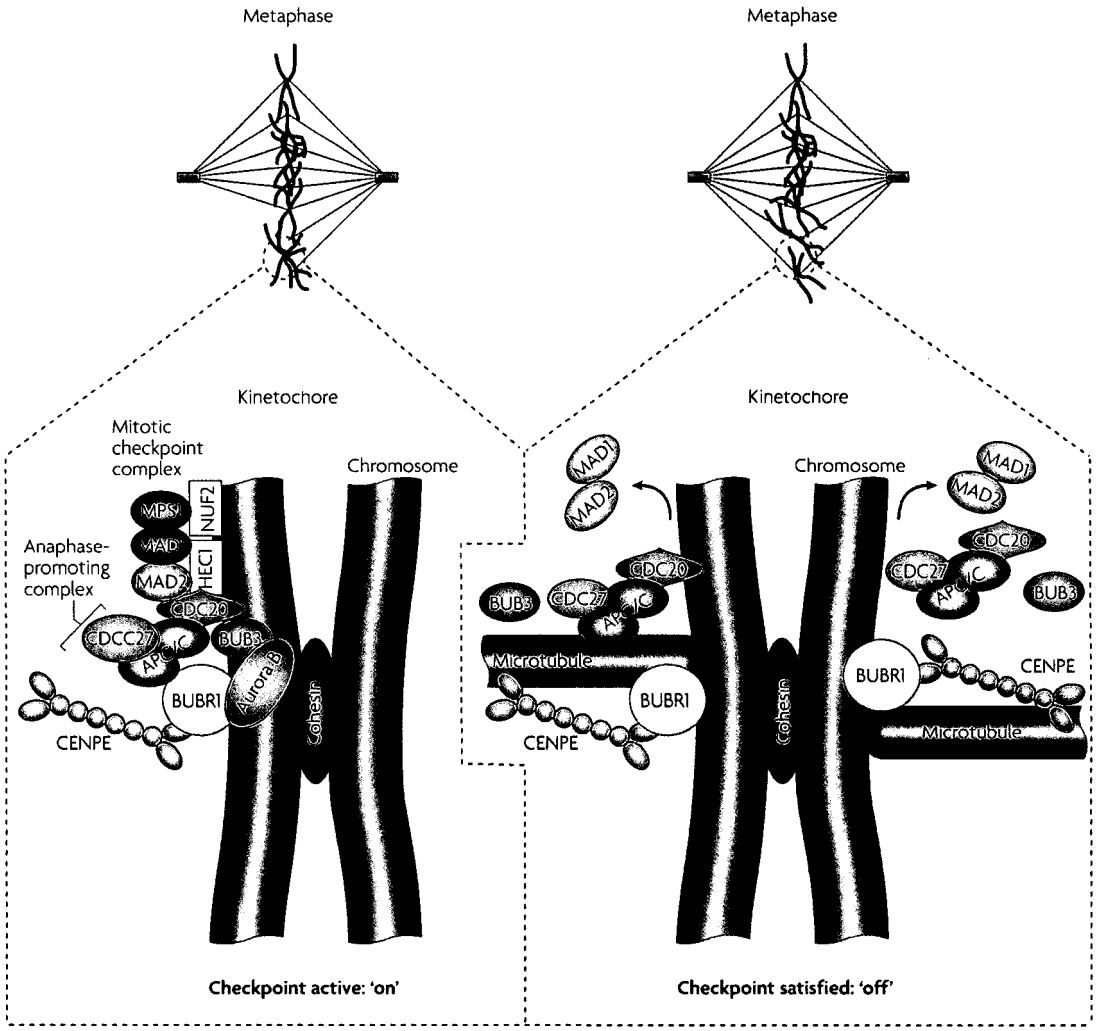

Figure 2 | **The mitotic checkpoint.** Crucial regulators of the mitotic checkpoint at the kinetochore are shown. On each kinetochore, when microtubules are captured and tension in these is detected, the checkpoint is satisfied and MAD2 is released, providing the biochemical signal to proceed to anaphase. Further detail on checkpoint regulation has been previously reviewed[80]. CDC, cell division cycle; CENPE, centromere protein E; MAD, mitotic arrest deficient.

Overexpression of PLK1 seems to be strongly associated with cancer. Several studies have shown correlations between PLK1 expression, histological grade and poor prognosis in several types of cancer[51]. PLK1 might have a role in oncogenesis through its regulation of tumour suppressors such as p53 and BRCA2 (REF. 52). The inhibition of PLK1 by small molecules or siRNA has been shown to interfere with several stages of mitosis[53,54].Therefore, targeting PLK1 offers an opportunity to treat cancer with a targeted anti-mitotic approach that will inhibit several important regulatory events in tumour cells.

Agents in clinical development

KSP inhibitors. One of the first targeted anti-mitotic agents, and the first KSP inhibitor to be studied in the clinic, was SB-715992; also known as ispinesib. This agent is a small-molecule inhibitor of the KSP ATPase, and is uncompetitive with ATP and ADP. It is very specific for KSP, and is 40,000 times more selective for KSP than any for other kinesins[27]. It was initially evaluated on two schedules by intravenous administration — once every 21 days[55,56] or on day 1, 8 and 15 every 28 days[57]. The cumulative dose delivered on both schedules was similar. The dose-limiting toxicity on both schedules was neutropaenia, with relative sparing of the remaining haematopoietic lineages. Other toxicities included mild to moderate gastrointestinal effects including nausea, vomiting and diarrhoea. During dose escalation, pharmacodynamic activity was shown in several patients on the basis of increased levels of phospho-histone-H3 in serial tumour biopsies, consistent with the mitotic arrest of tumour cells after dosing. This pharmacodynamic effect was not sufficient to result in clinical activity in all cases. Prolonged stable disease in excess of 6 months was observed on both schedules in several patients with different tumour types, including some that are usually not responsive to anti-tubulin agents, such as renal-cell, hepatocellular and colorectal carcinomas. This agent

Pharmacodynamic
Measurable physiological changes that occur in response to pharmacological modulation.

is currently in phase II development, and its activity is being evaluated in several tumour types including breast, non-small-cell lung, ovarian, colorectal, head and neck, melanoma, hepatocellular, androgen-independent prostate and renal cell. So far it has shown activity as a single agent in patients with metastatic breast cancer who have relapsed and/or progressed following treatment with an anthracycline and a taxane[58].

The second KSP ATPase inhibitor to undergo clinical evaluation, SB-743921, was derived from a distinct chemical series, but is similar to ispinesib, albeit more potent, in the spectrum of pre-clinical anti-tumour activity[59]. It is in phase I trials at present, and is associated with dose-limiting toxicities that include neutropaenia, increased activity of hepatic enzymes and hyperbilirubinaemia[60,61]. One individual with cholangiocarcinoma achieved a partial response, and two others experienced prolonged (>6 months) stable disease.

MK-0731 is another potent small-molecule inhibitor of KSP ATPase activity with >20,000 fold selectivity for KSP over other kinesins. It is currently undergoing phase I evaluation as a 24 hour intravenous infusion every 21 days[62]. The main dose-limiting toxicities include neutropaenia and increased activity of hepatic transaminases. Prolonged stable disease is the best response reported so far. Dose escalation is ongoing.

Table 3 | **Clinical results with new mitotic-targeted agents**

Target and anti-mitotic agent	Phase of clinical development	Number of patients treated	Duration and route of administration and schedule(s) evaluated	Clinical results	DLTs/significant adverse Events
Kinesin spindle protein (KSP)					
SB-715992 (ispinesib)	I/II	200+	1 hour intravenous; D1 Q21; D1, 8, 15 Q28; D1–3 Q21	Prolonged SD observed in phase I in several tumour types; ~10% of patients with metastatic breast cancer relapsing after anthracycline and taxane treatment achieved a PR	Prolonged neutropaenia, neutropaenia with fever/ infection
SB-743921	I	44	1 hour intravenous; D1 Q21	PR observed in a patient with cholangiocarcinoma	Prolonged neutropaenia, neutropaenia with fever/infection, hepatic enzyme elevation and hyperbilirubinaemia
MK-0731	I	17	24 hour intravenous; D1 Q21	Prolonged SD observed in several patients	Prolonged neutropaenia, hepatic enzyme elevation
Polo-like kinase1 (Plk1)					
BI 2536	I	104	1 hour intravenous; D1 Q21; D1–3 Q21; D1, 8 Q21	Transient PR seen in patients with head and neck cancer dosed on the D1 Q21 schedule; cumulative dose similar regardless of schedule	Prolonged neutropaenia, neutropaenia with fever/infection, thrombocytopaenia, nausea, vomiting and fatigue
ON 01910	I	18	2 hour intravenous; D1, 4, 8, 11, 15, and18 Q28; 24 hour intravenous D1–3 Q14	Prolonged SD observed on 24 hour infusion schedule	Mild to moderate neutropaenia, anaemia, hepatic enzyme elevations, gastro-intestinal symptoms, mild fatigue
Aurora kinase inhibitors					
MK-0457 (VX-680); Aurora A, B and C inhibitor	I/II	22+	24 hour intravenous; D1 Q21; D1–5 Q28; D1–5 Q21	Prolonged SD on D1–5 Q28 in solid tumours; phase II studies include CRC and NSCLC; evaluating imatinib-resistant patients with T315I mutant who are also resistant to dasatinib	Prolonged neutropaenia; neutropaenia with fever/ infection
AZD1152; Aurora B inhibitor	I/II	19+	2 hour intravenous; D1, 8, 15 Q21; D1,8 Q28; D1, 2 Q14; 24 hour intravenous; D1, 2 Q14	Prolonged SD in solid tumour patients; evaluation of AML ongoing	Prolonged neutropaenia; neutropaenia with fever/ infection

AML, acute myeloid leukaemia; CRC, colorectal cancer; D, day; NSCLC, non-small cell lung-cancer; SD, stable disease; PR, partial response; Q, every.

Box 1 | **Aurora kinases**

There are three Aurora kinase family members in mammalian cells, Aurora A, B and C; Aurora A is more evolutionarily distant from the very closely linked B and C members. Aurora A and B are ubiquitously expressed in all dividing cells, whereas Aurora C expression seems to be restricted to the testes[3].

Aurora A accumulates and peaks in abundance and kinase activity at the G2/M phase of the cell cycle, where it has a crucial role in centrosome maturation and separation during early prophase. The Aurora A gene copy number, mRNA and protein levels are found to be increased in many human epithelial tumour types including breast, bladder, colon, gastric, hepatocellular, ovarian and pancreatic carcinomas, with increasing severity of disease frequently associated with increased expression[3]. Aurora A can function as an oncogene in the transformation of rodent fibroblast cells and has also been described as a low-penetrance tumour-susceptibility gene. Substrates of Aurora A of significant interest include the tumour suppressor p53, and BRCA1, a breast-cancer-associated tumour suppressor.

Aurora B also accumulates during the lead-up to mitosis, with peak kinase activity occurring slightly later than Aurora A. Aurora B is a member of the chromosomal passenger complex (CPC), and is involved in histone H3 phosphorylation, chromosomal condensation, chromosomal alignment on the metaphase plate, bi-polar centromere-microtubule attachments, spindle checkpoint and cytokinesis. Much less is known about Aurora C function; however, recent studies indicate that Aurora C might have a role similar to Aurora B, although it is normally expressed at high levels in the testes and might have a specific role in male meiosis.

As clinical experience with KSP inhibitors accumulates, some patterns are emerging with regards to the adverse-event profile. The common dose-limiting toxicities observed (neutropaenia and increased activity of hepatic transaminases) are similar to some of the tubulin-targeting drugs (TABLE 1). However, some clear distinctions are also becoming apparent. For example, clinically significant neuropathy, alopecia and mucositis, which are common side effects of tubulin-targeting drugs, are rarely observed for any of the three KSP inhibitors described above. In addition, nausea and vomiting (mild–moderate severity) occurred infrequently with these inhibitors.

Aurora and PLK inhibitors. MK-0457, also known as VX-680, is the first aurora kinase inhibitor to enter clinical trials[63]. It is an ATP-competitive Aurora A, B and C inhibitor with nM potency for the inhibition of cell proliferation in culture. In addition, MK-0457 potently inhibits FMS-related tyrosine kinase 3 (FLT3), and was recently shown to inhibit several imatinib-resistant mutant forms of ABL kinase, including the T315I mutant that is resistant to both imatinib and dasatinib. It is in phase I and II trials at present (TABLE 3). Various protracted intravenous infusions were tested in phase I trials for patients with solid and haematological malignancies. The main dose-limiting toxicity in the solid-tumour study was neutropaenia. Evidence for a pharmacodynamic response in the skin was sought during dose escalation through assessments of levels of phospho-histone-H3 (PHH3), Ki67 and cyclin B1 expression before and after dosing in the solid-tumour study. Despite evidence of haematological toxicity in these patients, no significant evidence of mitotic delay or arrest, or decrease in proliferation was noted in the skin biopsies. In this case, although there was evidence of drug effect on proliferating cells (that is, haematopoietic cells), there was no evidence of such an effect in the skin for these particular cases. As a pan-aurora inhibitor is expected to cause premature mitotic exit without cytokinesis resulting in aneuploidy, mitotic arrest is not expected with this particular agent, although a delay in mitotic progression is possible based on preclinical observations. In addition, a decrease in PHH3 should occur based on Aurora B inhibition, which is distinct from the other anti-mitotics discussed in this Review that are associated with an increase in this marker. The challenge is to develop an assay capable of detecting a decrease in the level of this phosphoprotein, especially if pretreatment baseline values in tumours are low. Furthermore, the samples were obtained from skin and might not be representative of the tumour response. Prolonged stable disease (≥6 months) was noted in two patients with solid tumours. Phase II studies are ongoing or planned in colorectal cancer, non-small-cell lung cancer and acute myeloid leukaemia.

AZD1152, is a selective ATP-competitive Aurora B inhibitor, with an IC_{50} for cellular proliferation of 5–35 nM. Several schedules are being explored in phase I trials (TABLE 3) with a plan to evaluate pharmacodynamic tumour markers of response in an expanded cohort using the best schedule[64]. So far the main dose-limiting toxicity for the weekly 2 hour intravenous infusion has been neutropaenia. Further schedule evaluation is ongoing. A phase I–II study in acute myeloid leukaemia is ongoing in addition to the phase I solid-tumour studies.

The first ATP-competitive inhibitor of PLK1, BI 2536, is currently in phase I development. This small-molecule inhibitor was evaluated on three separate schedules of intravenous administration[65,66] (TABLE 3). A similar toxicity profile and cumulative dose delivered were observed regardless of the schedule evaluated. The dose-limiting toxicity was neutropaenia, with the addition of thrombocytopaenia observed on the day 1–3 schedule.

ON 01910 is an ATP non-competitive inhibitor of PLK1 (IC_{50} 9–10nM) that probably interferes with the ability of PLK to bind its substrates. In addition, it has low nM potency against ABL, FLT1 and platelet derived growth factor receptor (PDGFR). It is currently in phase I trials undergoing evaluation of 2 different dosing schedules[67,68] (TABLE 3). Adverse events related to therapy include mild to moderate anaemia, leukopaenia, increased activity of hepatic enzymes, gastrointestinal symptoms and fatigue. Dose escalation continues on both schedules.

Neuropathy
A pathological state in which the normal function of the peripheral nervous system is perturbed. In the case of tubulin-binding drugs, this typically manifests as the inhibition of sensory neurons resulting in tingling and/or loss of feeling.

IC_{50}
The concentration of a an inhibitor that is required for 50% inhibition of its target *in vitro*.

Box 2 | **Polo-like kinases**

In mammalian cells there are four polo-like kinases, PLK1, PLK2, PLK3 and PLK4. *PLK2* and *PLK3* are immediate early genes, suggesting that both have roles outside mitosis[4]. PLK2 is expressed primarily in the G1 phase of the cell cycle, and the expression of PLK3 does not vary with the cell cycle. Much less is known about PLK4.

PLK1 is highly expressed in cells with a high mitotic index. The expression and activity of PLK1 increases during the cell cycle, reaching its peak during mitosis when it is also maximally phosphorylated. The polobox domain facilitates specific interaction with phospho-epitopes, many of which are created by cyclin dependent kinase 1 (CDK1). This localizes PLK1 to specific complexes where it can phosphorylate many substrates. Among the known substrates, PLK1 phosphorylates and activates the phosphatase cell division cycle 25C (CDC25C), which in turn removes inhibitory phosphates from CDK1. This sets up an activation loop for CDK1 that leads to mitotic entry. PLK1 also phosphorylates cyclin B1, the cyclin partner of CDK1, resulting in nuclear localization where it binds to CDK1. During mitosis, PLK1 has been shown to have roles in centrosome maturation and microtubule dynamics involved in the formation of the mitotic spindle. PLK1 is also involved in the exit of cells from mitosis by phosphorylating and activating subunits of the anaphase-promoting complex (CDC16 and CDC27). PLK1 also phosphorylates cohesin proteins that hold sister chromatids together, exposing separase cleavage sites and enabling the separation of sister chromatids during anaphase. PLK1 might also have a role in cytokinesis through phosphorylation of the kinesin-like motor protein MKLP1.

Based on early clinical experience with Aurora and PLK inhibitors, the adverse-event profile suggests that the main dose-limiting toxicity for these agents is neutropaenia, with no clinically significant neuropathy observed so far.

Anti-mitotic drugs and mechanisms of cell death

Since the discovery of tubulin-targeting agents decades ago, tumour cells have been widely regarded as being very sensitive to mitotic arrest, and often undergo cell death in response to agents that perturb the mitotic spindle. Nevertheless, a solid molecular explanation of how cells die in response to mitotic checkpoint activation has not emerged. This remains a significant challenge for the development of new mitosis-selective drugs. All the targets covered in this Review are essential for proper mitotic division in all cells, including both tumour and normal cells. Unlike the oncogene targets involved in signal transduction and transcriptional regulation, the mitotic-selective targets have not been conclusively shown to have a cancer-specific gain of function in mitosis that make tumour cells more dependent on them compared with normal proliferating cells. Although there are many reports showing that mitotic targets are highly expressed in tumour tissue compared with normal tissue, and that there is also a correlation between high expression levels and poor prognosis, these observations might simply be a reflection of the higher numbers of proliferating cells in the tumour tissue, as these targets are cell-cycle regulated. Furthermore, the correlation of high mitosis-selective target expression with poor prognosis is an extension of the prognostic use of the Ki67 antigen. More aggressive tumours have more cell proliferation occurring in the tumour, and therefore more cells that express cell-cycle genes such as *KI67* and mitotic targets. Therefore, the high expression of mitotic targets in cancer is more likely to be a result of the disease than a cause of the disease. These targets are still promising points of intervention in cancer, despite not being disease-causing, because they are essential for tumour cell proliferation. Effects on normal cell proliferation are to be expected, but these have proven to be manageable in the clinic for the cytotoxic chemotherapies that are in use today. The basis for the therapeutic index for anti-mitotic drugs might lie downstream of the mitotic checkpoint and be manifested in how certain tumour cells respond to the mitotic injury caused by these inhibitors. It might be important to view these targets as a means to perturb the mitotic process in specific ways. Whether the cells die or escape from this perturbation results from the downstream response to it.

Studies using various anti-mitotic drugs against tumour cell lines have shown that there are various ways that different cells can respond to the same mitotic injury. In the case of KSP, Tao *et al.*[9] have shown that HCT116 colon carcinoma cells undergo mitotic arrest, followed by mitotic slippage and cell death following treatment with a KSP inhibitor. In this case, cell death was characterized by caspase 3 activation and could be prevented with a caspase inhibitor and therefore seems to involve classic apoptotic pathways. However, HT29 colon carcinoma cells treated with the same compound underwent mitotic arrest but did not progress to cell death. This suggests that the factors that determine whether cell death will occur lie downstream of the primary effect (prometaphase arrest) of this compound. In another study with KSP inhibitors[11], Marcus *et al.* showed that apoptosis involving poly(ADP ribose) polymerase (PARP) cleavage and caspases 8 and 9 ocurred after mitotic arrest in an ovarian cancer cell line.

Apoptosis is not the only mechanism by which cells die following a failed mitosis. Many studies have described a form of cell death called mitotic catastrophe[12]. This form of cell death does not require caspase 9 or 3 and can still occur in the presence of caspase inhibitors such as z-VAD-fmk. It requires CDK1 activity and is characterized by fragmentation of the nucleus to give micronuclei, and can be associated with the activation of caspase 2, a caspase not involved in the classic apoptosis pathway. DNA damage, tubulin agents and PLK1 inhibition have all been reported to cause mitotic catastrophe in some cell lines. Nevertheless, there is no molecular explanation for how mitotic catastrophe is initiated or regulated. There are also reports of both apoptotic cell death and mitotic catastrophe in response to PLK1 inhibition. Steegmaier *et al.*[69] used a small-molecule inhibitor of PLK1 on HeLa cells, and reported mitotic arrest followed by apoptosis with PARP cleavage and DNA fragmentation. In another study in HeLa cells using dominant-negative PLK1, Cogswell *et al.* reported the induction of cell death through mitotic catastrophe[70]. The rapid induction of cell death by exposure to the Aurora inhibitor VX-680 was shown to require a compromised p53-dependent postmitotic checkpoint in one model system[47]. The authors showed that endoreduplication followed by apoptosis occurs more significantly in cells with abrogated p53 pathway function, as shown by the lack of p21 induction. These observations are consistent with those obtained with another pan-Aurora kinase

Immediate early genes
Genes whose expression is induced rapidly by growth factor stimulation.

Mitotic slippage
When a cell exits from mitosis without successfully separating the chromosomes or undergoing cytokinesis it is said to have undergone mitotic slippage. Essentially, such a cell has slipped out of mitosis and entered a G1-like cell-cycle state but it now has double the chromosome number that it should have in G1.

Endoreduplication
This occurs after a cell undergoes mitotic slippage (having not divided), and then proceeds through the G1 phase with 4N DNA content and enters S phase to replicate its DNA again.

inhibitor, PHA-680632, where normal human dermal fibroblast cells did not undergo extensive endoreduplication or apoptosis[71]. Therefore, there are several ways that cell lines respond to the inhibition of the various mitotic targets: stable mitotic arrest, apoptosis, mitotic catastrophe, mitotic slippage followed by cell death, mitotic slippage followed G1 growth arrest or mitotic slippage followed by the continuation of cell cycle and endoreduplication. Although there have been studies that define these responses in certain cell lines, a broadly applicable mechanistic marker that predicts how a tumour cell will respond to any particular anti-mitotic therapy has yet to be identified.

Recent efforts have begun to identify expression profiles that predict response to anti-mitotic drugs. Although such studies do not provide a mechanistic explanation for response, they have shown that the prediction of response is feasible. Using clinical biopsies, Chang *et al*[72] analysed gene-expression profiles and identified a 92-gene set that could predict with ~90% accuracy whether the tumour would respond to neoadjuvant docetaxel treatment. Subsequently, other studies have also been able to correlate specific gene-expression patterns with response to taxane-containing regimens[73]. These studies all used clinical tissue specimens and focused on tumour types and disease settings in which the taxanes were already known to have activity. Agents directed at the new mitosis-selective targets have the additional challenge that responsive tumour types have yet to be identified in the clinic. Therefore, it will be necessary to rely on preclinical models to identify the predictive markers to help target these agents to specific patients. There are some early examples of finding predictive markers with preclinical systems[74], but whether these markers will also be predictive in the clinic remains to be proven.

Drug combination approaches

Most current cancer treatments are combinations of chemotherapeutic agents, and it is expected that newer targeted anti-mitotic agents will achieve their greatest efficacy in combination with traditional cytotoxic agents and emerging targeted therapies. Taxanes and the vinca alkaloids have been combined with nearly every class of cytotoxic chemotherapy for the treatment of various tumour types with varying degrees of success. One objective of combination therapy is to improve efficacy at the same time as maintaining acceptable overall toxicity. This is often achieved by combining agents with non cross-resistant mechanisms of action and different spectrums of toxicity. Taxanes are commonly used in combination with platinum analogues for non-small-cell lung cancer and with anthracyclines for breast cancer. The paclitaxel plus doxorubicin combination was associated with increased incidence of cardiotoxicity compared with doxorubicin alone[75]; however, this was successfully managed by altering the dosing schedule of the two drugs to overcome a pharmacokinetic interaction associated with increased doxorubicin exposure[76]. One strategy for the newer targeted anti-mitotic agents is to see if there is any benefit to the addition of these new agents with current first-line and second-line treatment regimens. A significant challenge to this approach will be to manage the expected neutropaenia from these new targeted anti-mitotic agents in combination with existing therapies that are myelosuppressive. However, the future holds great promise, with many new targeted agents currently in the development pipeline, and there might be some rational approaches to prioritize potential combinations with the new targeted anti-mitotic agents reviewed here. *In vitro* studies have shown that tumour cells treated with anti-mitotic agents frequently, but not always, result in cell death. Tumour cells that arrest and survive through a mitotic block must have developed strong cell-survival mechanisms to become resistant to cell death. New targeted approaches that block these survival mechanisms could potentially provide benefits with the anti-mitotic agents, old and new. For example, the Akt pathway is an important regulator of cell survival, and the activity of this pathway is often increased in cancer cells. It has been shown in *in vitro* and *in vivo* studies that the combination of paclitaxel with rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), just downstream from Akt, is dramatically synergistic on rapamycin-sensitive breast cancer cells[77]. Agents that target various points along the Akt pathway are in the preclinical phase of development, and might provide benefits in combination with current and emerging anti-mitotic agents.

Challenges in developing new anti-mitotic drugs

Although the new generation of anti-mitotics holds much promise, there are still many challenges posed by their clinical development. To begin, what is the most appropriate way to develop these new anti-mitotics? Should they be viewed as 'taxane-like' but with the potential to avoid the neurotoxicity typical of anti-tubulin agents? Will these agents have broad activity in tumour types historically sensitive to anti-tubulin agents, or in tumours that have become resistant to anti-tubulin agents, and will they have an improved safety profile? Alternatively, given that the target for these new anti-mitotics is only expressed during mitosis, in clear distinction to tubulin, which has important functions outside the cell cycle including vesicle transport and cell signalling, will the newer agents have a more limited and distinct spectrum of efficacy? If so, will it be possible to identify molecular phenotype(s) associated with increased sensitivity to these new agents to aid in patient selection?

The diminished risk of neurotoxicity posed by the newer agents is supported by the clinical trial data. In fact, the primary dose-limiting toxicity for many of the new targeted anti-mitotics seems to be neutropaenia, with relative sparing of the other haematopoetic lineages. With regards to clinical activity, it is too early to tell for most of the agents in development, although ispinesib has shown some activity in taxane-exposed patients with metastatic breast cancer in a phase II study.

A unique challenge for the development of these targeted anti-mitotics is the fact that there is no clear understanding of the link between the inhibition of the respective mitotic target and subsequent cell death as discussed above. For example, some of the targeted

anti-mitotic agents, such as KSP and PLK1 inhibitors, induce mitotic arrest by activating the mitotic checkpoint. It is unclear whether a minimum duration of mitotic arrest is required, or whether mitotic arrest needs to be coupled with mitotic slippage for subsequent cell death to occur. These requirements might differ for each anti-mitotic agent and for a given tumour.

Another challenge facing the development of these agents is how to identify the patients who might have the best chance of responding to these agents. Success in this area will probably depend on approaches that have already been applied to predicting response to taxane therapy, as discussed earlier.

To aid the clinical development of these agents, it will be crucial to assess the effect of these agents on their respective target(s) within the tumour. Selecting potential surrogate tissues to assess pharmacodynamic response with these agents is uniquely challenging. In addition to the usual caveats about the relevance of such tissues to what is occurring within tumours, there is the concern that the targets for many of these anti-mitotic agents will be absent in most surrogate tissues, such as peripheral blood mononucleocytes or skin, owing to low or absent proliferation. Such pharmacodynamic assessments should be consistent with the phenotype that is known to be associated with target inhibition, as discussed above. In addition, it might be important not only to assess pharmacodynamic effects in tumour tissue, but also to assess the duration of such an effect, as both might be crucial determinants of a relevant downstream biological response (such as apoptosis). Pharmacodynamic tumour marker evaluation at the maximum tolerated dose (MTD; and below the MTD if possible) can provide insights into a potential therapeutic window by verifying target inhibition in the tumour at such doses. This might be aided by the incorporation of serial tumour biopsies and functional imaging (such as fluorothymidine-positron emission topography for agents that induce cell-cycle arrest)[78,79]. Furthermore, it might be important to adequately explore alternative schedules if pharmacokinetic and pharmacodynamic data indicate that increased target inhibition or an improved therapeutic window might be achieved on a different schedule.

It is clear that we are entering a new era in anti-mitotic therapy with the identification and now clinical translation of new targets in mitosis beyond tubulin. Whether we will improve on the tubulin agents remains to be seen. The challenge lies in improving our understanding of the important links between disrupting key components of the mitotic machinery and the apoptotic pathway. Verifying target inhibition in the tumour, optimizing dose and schedule with an eye towards the therapeutic window, and identifying potential determinants of sensitivity must all be taken into consideration during clinical development, and will ultimately help determine the potential role for these agents in the advancement of cancer treatment.

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Competing interests statement
The authors declare competing financial interests. See web version for details.

DATABASES

The following terms in this article are linked online to:
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TAXOL®
(paclitaxel) INJECTION
(Patient Information Included)

℞ ONLY

WARNING

TAXOL® (paclitaxel) Injection should be administered under the supervision of a physician experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2%-4% of patients receiving TAXOL in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H_2 antagonists. (See **DOSAGE AND ADMINISTRATION.**) Patients who experience severe hypersensitivity reactions to TAXOL should not be rechallenged with the drug.

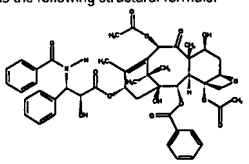
TAXOL therapy should not be given to patients with solid tumors who have baseline neutrophil counts of less than 1,500 cells/mm³ and should not be given to patients with AIDS-related Kaposi's sarcoma if the baseline neutrophil count is less than 1000 cells/mm³. In order to monitor the occurrence of bone marrow suppression, primarily neutropenia, which may be severe and result in infection, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving TAXOL.

DESCRIPTION

TAXOL® (paclitaxel) Injection is a clear colorless to slightly yellow viscous solution. It is supplied as a nonaqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. TAXOL is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL* (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.

Paclitaxel is a natural product with antitumor activity. TAXOL is obtained via a semi-synthetic process from *Taxus baccata*. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

Paclitaxel has the following structural formula:



Paclitaxel is a white to off-white crystalline powder with the empirical formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.9. It is highly lipophilic, insoluble in water, and melts at around 216-217°C.

CLINICAL PHARMACOLOGY

Paclitaxel is a novel antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. In addition, paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Following intravenous administration of TAXOL, paclitaxel plasma concentrations declined in a biphasic manner. The initial rapid decline represents distribution to the peripheral compartment and elimination of the drug. The later phase is due, in part, to a relatively slow efflux of paclitaxel from the peripheral compartment.

Pharmacokinetic parameters of paclitaxel following 3- and 24-hour infusions of TAXOL at dose levels of 135 and 175 mg/m² were determined in a Phase 3 randomized study in ovarian cancer patients and are summarized in the following table:

Table 1: Summary of Pharmacokinetic Parameters - Mean Values

Dose (mg/m²)	Infusion Duration (h)	N (patients)	C_{max} (ng/mL)	AUC (0-∞) (ng·h/mL)	T-HALF (h)	CL_T (L/h/m²)
135	24	2	195	6300	52.7	21.7
175	24	4	365	7993	15.7	23.8
135	3	7	2170	7952	13.1	17.7
175	3	5	3650	15007	20.2	12.2

C_{max} = Maximum plasma concentration
AUC (0-∞) = Area under the plasma concentration-time curve from time 0 to infinity
CL_T = Total body clearance

It appeared that with the 24-hour infusion of TAXOL, a 30% increase in dose (135 mg/m² versus 175 mg/m²) increased the C_{max} by 87%, whereas the AUC (0-∞) remained proportional. However, with a 3-hour infusion, for a 30% increase in dose, the C_{max} and AUC (0-∞) were increased by 68% and 89%, respectively. The mean apparent volume of distribution at steady state, with the 24-hour infusion of TAXOL, ranged from 227 to 688 L/m², indicating extensive extravascular distribution and/or tissue binding of paclitaxel.

The pharmacokinetics of paclitaxel were also evaluated in adult cancer patients who received single doses of 15-135 mg/m² given by 1-hour infusions (n=15), 30-275 mg/m² given by 6-hour infusions (n=36), and 200-275 mg/m² given by 24-hour infusions (n=54) in Phase 1 & 2 studies. Values for CL_T and volume of distribution were consistent with the findings in the Phase 3 study. The pharmacokinetics of TAXOL in patients with AIDS-related Kaposi's sarcoma have not been studied.

In vitro studies of binding to human serum proteins, using paclitaxel concentrations ranging from 0.1 to 50 µg/mL, indicate that between 89%-98% of drug is bound; the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine did not affect protein binding of paclitaxel.

* Cremophor® EL is the registered trademark of BASF Aktiengesellschaft. Cremophor® EL is further purified by a Bristol-Myers Squibb Company proprietary process before use.

After intravenous administration of 15-275 mg/m² doses of TAXOL (paclitaxel) Injection as 1-, 6-, or 24-hour infusions, mean values for cumulative urinary recovery of unchanged drug ranged from 1.3% to 12.6% of the dose, indicating extensive non-renal clearance. In five patients administered a 225 or 250 mg/m² dose of radiolabeled TAXOL as a 3-hour infusion, a mean of 71% of the radioactivity was excreted in the feces in 120 hours, and 14% was recovered in the urine. Total recovery of radioactivity ranged from 56% to 101% of the dose. Paclitaxel represented a mean of 5% of the administered radioactivity recovered in the feces, while metabolites, primarily 6α-hydroxypaclitaxel, accounted for the balance. *In vitro* studies with human liver microsomes and tissue slices showed that paclitaxel was metabolized primarily to 6α-hydroxypaclitaxel by the cytochrome P450 isozyme CYP2C8; and to two minor metabolites, 3'-*p*-hydroxypaclitaxel and 6α, 3'-*p*-dihydroxypaclitaxel, by CYP3A4. *In vitro*, the metabolism of paclitaxel to 6α-hydroxypaclitaxel was inhibited by a number of agents (ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, teniposide, etoposide, and vincristine), but the concentrations used exceeded those found *in vivo* following normal therapeutic doses. Testosterone, 17α-ethinyl estradiol, retinoic acid, and quercetin, a specific inhibitor of CYP2C8, also inhibited the formation of 6α-hydroxypaclitaxel *in vitro*. The pharmacokinetics of paclitaxel may also be altered *in vivo* as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4. (See **PRECAUTIONS: Drug Interactions.**)

The disposition and toxicity of paclitaxel 3-hour infusion were evaluated in 35 patients with varying degrees of hepatic function. Relative to patients with normal bilirubin, plasma paclitaxel exposure in patients with abnormal serum bilirubin ≤2 times upper limit of normal (ULN) administered 175 mg/m² was increased, but with no apparent increase in the frequency or severity of toxicity. In five patients with serum total bilirubin >2 times ULN, there was a statistically nonsignificant higher incidence of severe myelosuppression, even at a reduced dose (110 mg/m²), but no observed increase in plasma exposure. (See **PRECAUTIONS: Hepatic** and **DOSAGE AND ADMINISTRATION.**) The effect of renal dysfunction on the disposition of paclitaxel has not been investigated.

Possible interactions of paclitaxel with concomitantly administered medications have not been formally investigated.

CLINICAL STUDIES

Ovarian Carcinoma:

First-Line Data- The safety and efficacy of TAXOL followed by cisplatin in patients with advanced ovarian cancer and no prior chemotherapy were evaluated in two Phase 3 multicenter, randomized, controlled trials. In an Intergroup study led by the European Organization for Research and Treatment of Cancer involving the Scandinavian Group NOCOVA, the National Cancer Institute of Canada, and the Scottish Group, 680 patients with Stage IIb-c, III, or IV disease (optimally or non-optimally debulked) received either TAXOL 175 mg/m² infused over 3 hours followed by cisplatin 75 mg/m² (Tc) or cyclophosphamide 750 mg/m² followed by cisplatin 75 mg/m² (Cc) for a median of six courses. Although the protocol allowed further therapy, only 15% received both drugs for nine or more courses. In a study conducted by the Gynecological Oncology Group (GOG), 410 patients with Stage III or IV disease (>1 cm residual disease after staging laparotomy or distant metastases) received either TAXOL 135 mg/m² infused over 24 hours followed by cisplatin 75 mg/m² or cyclophosphamide 750 mg/m² followed by cisplatin 75 mg/m² for six courses.

In both studies, patients treated with TAXOL in combination with cisplatin had significantly higher response rate, longer time to progression, and longer survival time compared with standard therapy. These differences were also significant for the subset of patients in the Intergroup study with non-optimally debulked disease, although the study was not fully powered for subset analyses (Tables 2A and 2B). Kaplan-Meier survival curves for each study are shown in Figures 1 and 2.

Table 2A: Efficacy in the Phase 3 First-Line Ovarian Carcinoma Studies

	Intergroup (non-optimally debulked subset)		GOG-111	
	T175/3[a] c75 (n=218)	C750[a] c75 (n=227)	T135/24[a] c75 (n=196)	C750[a] c75 (n=214)
• **Clinical Response**[b]	(n=153)	(n=153)	(n=113)	(n=127)
- rate (percent)	58	43	62	48
- p-value[c]	0.016		0.04	
• **Time to Progression**				
- median (months)	13.2	9.9	16.6	13.0
- p-value[c]	0.0060		0.0008	
- hazard ratio (HR)[c]	0.76		0.70	
- 95% CI[c]	0.62-0.92		0.56-0.86	
• **Survival**				
- median (months)	29.5	21.9	35.5	24.2
- p-value[c]	0.0057		0.0002	
- hazard ratio[c]	0.73		0.64	
- 95% CI[c]	0.58-0.91		0.50-0.81	

[a] TAXOL dose in mg/m²/infusion duration in hours; cyclophosphamide and cisplatin doses in mg/m².
[b] Among patients with measurable disease only.
[c] Unstratified for the Intergroup Study, Stratified for Study GOG-111.

Table 2B: Efficacy in the Phase 3 First-Line Ovarian Carcinoma Intergroup Study

	T175/3[a] c75 (n=342)	C750[a] c75 (n=338)
• **Clinical Response**[b]	(n=162)	(n=161)
- rate (percent)	59	45
- p-value[c]	0.014	
• **Time to Progression**		
- median (months)	15.3	11.5
- p-value[c]	0.0005	
- hazard ratio[c]	0.74	
- 95% CI[c]	0.63-0.88	
• **Survival**		
- median (months)	35.6	25.9
- p-value[c]	0.0016	
- hazard ratio[c]	0.73	
- 95% CI[c]	0.60-0.89	

[a] TAXOL dose in mg/m²/infusion duration in hours; cyclophosphamide and cisplatin doses in mg/m².
[b] Among patients with measurable disease only.
[c] Unstratified.

Figure 1. Survival: Cc Versus Tc (Intergroup)

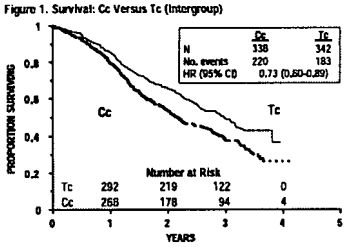
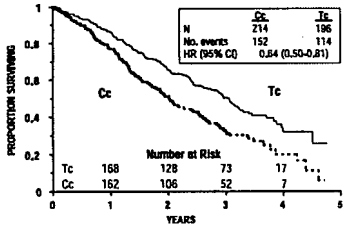


Figure 2. Survival: Cc Versus Tc (GOG-111)



The adverse event profile for patients receiving TAXOL (paclitaxel) Injection in combination with cisplatin in these studies was qualitatively consistent with that seen for the pooled analysis of data from 812 patients treated with single-agent TAXOL in 10 clinical studies. These adverse events and adverse events from the Phase 3 first-line ovarian carcinoma studies are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 11) and narrative form.

Second-Line Data- Data from five Phase 1 & 2 clinical studies (189 patients), a multicenter randomized Phase 3 study (407 patients), as well as an interim analysis of data from more than 300 patients enrolled in a treatment referral center program were used in support of the use of TAXOL in patients who have failed initial or subsequent chemotherapy for metastatic carcinoma of the ovary. Two of the Phase 2 studies (92 patients) utilized an initial dose of 135 to 170 mg/m² in most patients (>90%) administered over 24 hours by continuous infusion. Response rates in these two studies were 22% (95% CI: 11% to 37%) and 30% (95% CI: 18% to 46%) with a total of 6 complete and 18 partial responses in 92 patients. The median duration of overall response in these two studies measured from the first day of treatment was 7.2 months (range: 3.5-15.8 months) and 7.5 months (range: 5.3-17.4 months), respectively. The median survival was 8.1 months (range: 0.2-36.7 months) and 15.9 months (range: 1.8-34.5+ months).

The Phase 3 study had a bifactorial design and compared the efficacy and safety of TAXOL, administered at two different doses (135 or 175 mg/m²) and schedules (3- or 24-hour infusion). The overall response rate for the 407 patients was 16.2% (95% CI: 12.8% to 20.2%), with 6 complete and 60 partial responses. Duration of response, measured from the first day of treatment was 8.3 months (range: 3.2-21.6 months). Median time to progression was 3.7 months (range: 0.1+ - 25.1+ months). Median survival was 11.5 months (range: 0.2-26.3+ months).

Response rates, median survival, and median time to progression for the 4 arms are given in the following table.

Table 3: Efficacy in the Phase 3 Second-Line Ovarian Carcinoma Study

	175/3 (n=96)	175/24 (n=106)	135/3 (n=99)	135/24 (n=106)
• **Response**				
- rate (percent)	14.6	21.7	15.2	13.2
- 95% Confidence Interval	(8.5-23.6)	(14.5-31.0)	(9.0-24.1)	(7.7-21.5)
• **Time to Progression**				
- median (months)	4.4	4.2	3.4	2.8
- 95% Confidence Interval	(3.0-5.6)	(3.5-5.1)	(2.8-4.2)	(1.9-4.0)
• **Survival**				
- median (months)	11.5	11.8	13.1	10.7
- 95% Confidence Interval	(8.4-14.4)	(8.9-14.6)	(9.1-14.6)	(8.1-13.6)

Analyses were performed as planned by the bifactorial study design described in the protocol, by comparing the two doses (135 or 175 mg/m²) irrespective of the schedule (3 or 24 hours) and the two schedules irrespective of dose. Patients receiving the 175 mg/m² dose had a response rate similar to that for those receiving the 135 mg/m² dose: 18% vs. 14% (p=0.28). No difference in response rate was detected when comparing the 3-hour with the 24-hour infusion: 15% vs. 17% (p=0.50). Patients receiving the 175 mg/m² dose of TAXOL had a longer time to progression than those receiving the 135 mg/m² dose: median 4.2 vs. 3.1 months (p=0.03). The median time to progression for patients receiving the 3-hour vs. the 24-hour infusion was 4.0 months vs. 3.7 months, respectively. Median survival was 11.6 months in patients receiving the 175 mg/m² dose of TAXOL and 11.0 months in patients receiving the 135 mg/m² dose (p=0.92). Median survival was 11.7 months for patients receiving the 3-hour infusion of TAXOL and 11.2 months for patients receiving the 24-hour infusion (p=0.91). These statistical analyses should be viewed with caution because of the multiple comparisons made.

TAXOL remained active in patients who had developed resistance to platinum-containing therapy (defined as tumor progression while on, or tumor relapse within 6 months from completion of, a platinum-containing regimen) with response rates of 14% in the Phase 3 study and 31% in the Phase 1 & 2 clinical studies.

The adverse event profile in this Phase 3 study was consistent with that seen for the pooled analysis of data from 812 patients treated in 10 clinical studies. These adverse events and adverse events from the Phase 3 second-line ovarian carcinoma study are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 12) and narrative form.

The results of this randomized study support the use of TAXOL at doses of 135 to 175 mg/m², administered by a 3-hour intravenous infusion. The same doses administered by 24-hour infusion were more toxic. However, the study had insufficient power to determine whether a particular dose and schedule produced superior efficacy.

Breast Carcinoma:

Adjuvant Therapy- A Phase 3 intergroup study (Cancer and Leukemia Group B [CALGB], Eastern Cooperative Oncology Group [ECOG], North Central Cancer Treatment Group [NCCTG], and Southwest Oncology Group [SWOG]) randomized 3170 patients with node-positive breast carcinoma to adjuvant therapy with TAXOL (paclitaxel) Injection or to no further chemotherapy following four courses of doxorubicin and cyclophosphamide (AC). This multicenter trial was conducted in women with histologically positive lymph nodes following either a mastectomy or segmental mastectomy and nodal dissections. The 3 x 2 factorial study was designed to assess the efficacy and safety of three different dose levels of doxorubicin (A) and to evaluate the effect of the addition of TAXOL administered following the completion of AC therapy. After stratification for the number of positive lymph nodes (1-3, 4-9, or 10+), patients were randomized to receive cyclophosphamide at a dose of 600 mg/m² and doxorubicin at doses of either 60 mg/m² (on day 1), 75 mg/m² (in two divided doses on days 1 and 2), or 90 mg/m² (in two divided doses on days 1 and 2 with prophylactic G-CSF support and ciprofloxacin) every 3 weeks for four courses and either TAXOL 175 mg/m² as a 3-hour infusion every 3 weeks for four additional courses or no additional chemotherapy. Patients whose tumors were positive were to receive subsequent tamoxifen treatment (20 mg daily for 5 years); patients who received segmental mastectomies prior to study were to receive breast irradiation after recovery from treatment-related toxicities.

At the time of the current analysis, median follow-up was 30.1 months. Of the 2066 patients who were hormone receptor positive, 93% received tamoxifen. The primary analyses of disease-free survival and overall survival used multivariate Cox models, which included TAXOL administration, doxorubicin dose, number of positive lymph nodes, tumor size, menopausal status, and estrogen receptor status as factors. Based on the model for disease-free survival, patients receiving AC followed by TAXOL had a 22% reduction in the risk of disease recurrence compared to patients randomized to AC alone (Hazard Ratio [HR] = 0.78, 95% CI 0.67-0.91, p=0.0022). They also had a 26% reduction in the risk of death (HR = 0.74, 95% CI 0.60-0.92, p=0.0065). For disease-free survival and overall survival, p values were not adjusted for interim analyses. Kaplan-Meier curves are shown in Figures 3 and 4. Increasing the dose of doxorubicin higher than 60 mg/m² had no effect on either disease-free survival or overall survival.

Figure 3. Disease-Free Survival: AC Versus AC+T

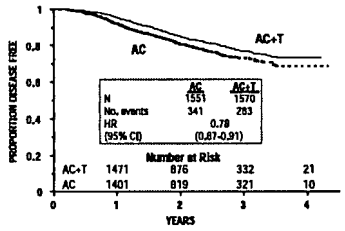
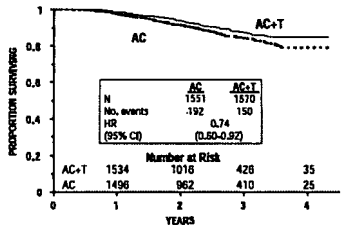


Figure 4. Survival: AC Versus AC+T



Subset analyses- Subsets defined by variables of known prognostic importance in adjuvant breast carcinoma were examined, including number of positive lymph nodes, tumor size, hormone receptor status, and menopausal status. Such analyses must be interpreted with care, as the most secure finding is the overall study result. In general, a reduction in hazard similar to the overall reduction was seen with TAXOL for both disease-free and overall survival in all of the larger subsets with one exception; patients with receptor-positive tumors had a smaller reduction in hazard (HR = 0.92) for disease-free survival with TAXOL than other groups. Results of subset analyses are shown in Table 4.

Table 4: Subset Analyses–Adjuvant Breast Carcinoma Study

Patient Subset	No. of Patients	Disease-Free Survival No. of Recurrences	Hazard Ratio (95% CI)	Overall Survival No. of Deaths	Hazard Ratio (95% CI)
• **No. of Positive Nodes**					
1-3	1449	221	0.72 (0.55-0.94)	107	0.76 (0.52-1.12)
4-9	1310	274	0.78 (0.61-0.99)	148	0.66 (0.47-0.91)
10+	360	129	0.93 (0.66-1.31)	87	0.90 (0.59-1.36)
• **Tumor Size (cm)**					
≤ 2	1096	153	0.79 (0.57-1.08)	67	0.73 (0.45-1.18)
> 2 and ≤ 5	1611	358	0.79 (0.64-0.97)	201	0.74 (0.56-0.98)
>5	397	111	0.75 (0.51-1.08)	72	0.73 (0.46-1.16)
• **Menopausal Status**					
Pre	1929	374	0.83 (0.67-1.01)	187	0.72 (0.54-0.97)
Post	1183	250	0.73 (0.57-0.93)	155	0.77 (0.56-1.06)
• **Receptor Status**					
Positive[a]	2066	293	0.92 (0.73-1.16)	126	0.83 (0.59-1.18)
Negative/Unknown[b]	1055	331	0.68 (0.55-0.85)	216	0.71 (0.54-0.93)

[a] Positive for either estrogen or progesterone receptors.
[b] Negative or missing for both estrogen and progesterone receptors (both missing: n=15).

These retrospective subgroup analyses suggest that the beneficial effect of TAXOL (paclitaxel) Injection is clearly established in the receptor-negative subgroup, but the benefit in receptor-positive patients is not yet clear. With respect to menopausal status, the benefit of TAXOL is consistent (see Table 4 and Figures 5-8).

Figure 5. Disease-Free Survival - Receptor Status Negative/Unknown AC Versus AC+T

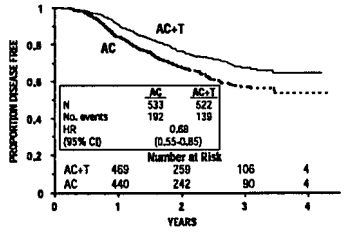


Figure 6. Disease-Free Survival - Receptor Status Positive AC Versus AC+T

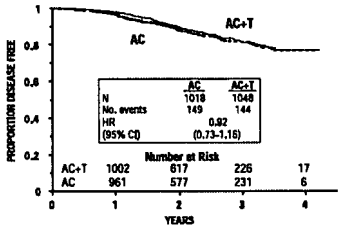


Figure 7. Disease-Free Survival - Premenopausal AC Versus AC+T

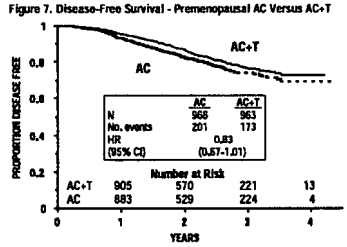
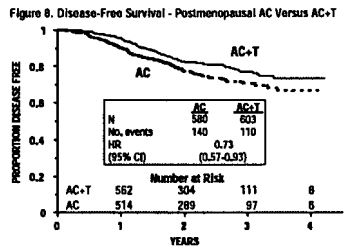


Figure 8. Disease-Free Survival - Postmenopausal AC Versus AC+T



The adverse event profile for the patients who received TAXOL subsequent to AC was consistent with that seen in the pooled analysis of data from 812 patients (Table 10) treated with single-agent TAXOL in 10 clinical studies. These adverse events are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 13) and narrative form.

After Failure of Initial Chemotherapy- Data from 83 patients accrued in three Phase 2 open label studies and from 471 patients enrolled in a Phase 3 randomized study were available to support the use of TAXOL in patients with metastatic breast carcinoma.

Phase 2 open label studies- Two studies were conducted in 53 patients previously treated with a maximum of one prior chemotherapeutic regimen. TAXOL was administered in these two trials as a 24-hour infusion at initial doses of 250 mg/m² (with G-CSF support) or 200 mg/m². The response rates were 57% (95% CI: 37% to 75%) and 52% (95% CI: 32% to 72%), respectively. The third Phase 2 study was conducted in extensively pretreated patients who had failed anthracycline therapy and who had received a minimum of two chemotherapy regimens for the treatment of metastatic disease. The dose of TAXOL was 200 mg/m² as a 24-hour infusion with G-CSF support. Nine of 30 patients achieved a partial response, for a response rate of 30% (95% CI: 15% to 50%).

Phase 3 randomized study- This multicenter trial was conducted in patients previously treated with one or two regimens of chemotherapy. Patients were randomized to receive TAXOL at a dose of either 175 mg/m² or 135 mg/m² given as a 3-hour infusion. In the 471 patients enrolled, 60% had symptomatic disease with impaired performance status at study entry, and 73% had visceral metastases. These patients had failed prior chemotherapy either in the adjuvant setting (30%), the metastatic setting (39%), or both (31%). Sixty-seven percent of the patients had been previously exposed to anthracyclines and 23% of them had disease considered resistant to this class of agents.

The overall response rate for the 454 evaluable patients was 26% (95% CI: 22% to 30%), with 17 complete and 99 partial responses. The median duration of response, measured from the first day of treatment, was 8.1 months (range: 3.4-18.1+ months). Overall for the 471 patients, the median time to progression was 3.5 months (range: 0.03-17.1 months). Median survival was 11.7 months (range: 0-18.9 months).

Response rates, median survival and median time to progression for the 2 arms are given in the following table.

Table 5: Efficacy in Breast Cancer after Failure of Initial Chemotherapy or Within 6 Months of Adjuvant Chemotherapy

	175/3 (n=235)	135/3 (n=236)
• **Response**		
- rate (percent)	28	22
- p-value	0.135	
• **Time to Progression**		
- median (months)	4.2	3.0
-p-value	0.027	
• **Survival**		
- median (months)	11.7	10.5
- p-value	0.321	

The adverse event profile of the patients who received single-agent TAXOL (paclitaxel) Injection in the Phase 3 study was consistent with that seen for the pooled analysis of data from 812 patients treated in 10 clinical studies. These adverse events and adverse events from the Phase 3 breast carcinoma study are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 14) and narrative form.

Non-Small Cell Lung Carcinoma (NSCLC)- In a Phase 3 open label randomized study conducted by the ECOG, 599 patients were randomized to either TAXOL (T) 135 mg/m² as a 24-hour infusion in combination with cisplatin (c) 75 mg/m², TAXOL (T) 250 mg/m² as a 24-hour infusion in combination with cisplatin (c) 75 mg/m² with G-CSF support, or cisplatin (c) 75 mg/m² on day 1, followed by etoposide (VP) 100 mg/m² on days 1, 2, and 3 (control).

Response rates, median time to progression, median survival, and one-year survival rates are given in the following table. The reported p-values have not been adjusted for multiple comparisons. There were statistically significant differences favoring each of the TAXOL plus cisplatin arms for response rate and time to tumor progression. There was no statistically significant difference in survival between either TAXOL plus cisplatin arm and the cisplatin plus etoposide arm.

Table 6: Efficacy Parameters in the Phase 3 First-Line NSCLC Study

	T135/24 c75 (n=198)	T250/24 c75 (n=201)	VP100[a] c75 (n=200)
• **Response**			
- rate (percent)	25	23	12
- p-value[b]	0.001	<0.001	
• **Time to Progression**			
- median (months)	4.3	4.9	2.7
- p-value[b]	0.05	0.004	
• **Survival**			
- median (months)	9.3	10.0	7.4
- p-value[b]	0.12	0.08	
• **One-Year Survival**			
- percent of patients	36	40	32

[a] Etoposide (VP) 100 mg/m² was administered I.V. on days 1, 2 and 3.
[b] Compared to cisplatin/etoposide.

In the ECOG study, the Functional Assessment of Cancer Therapy-Lung (FACT-L) questionnaire had seven subscales that measured subjective assessment of treatment. Of the seven, the Lung Cancer Specific Symptoms subscale favored the TAXOL 135 mg/m²/24 hour plus cisplatin arm compared to the cisplatin/etoposide arm. For all other factors, there was no difference in the treatment groups.

The adverse event profile for patients who received TAXOL in combination with cisplatin in this study was generally consistent with that seen for the pooled analysis of data from 812 patients treated with single-agent TAXOL in 10 clinical studies. These adverse events and adverse events from the Phase 3 first-line NSCLC study are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 15) and narrative form.

AIDS-Related Kaposi's Sarcoma- Data from two Phase 2 open label studies support the use of TAXOL as second-line therapy in patients with AIDS-related Kaposi's sarcoma. Fifty-nine of the 85 patients enrolled in these studies had previously received systemic therapy, including interferon alpha (32%), DaunoXome® (31%), DOXIL® (2%), and doxorubicin containing chemotherapy (42%), with 64% having received prior anthracyclines. Eighty-five percent of the pretreated patients had progressed on, or could not tolerate, prior systemic therapy.

In Study CA139-174 patients received TAXOL at 135 mg/m² as a 3-hour infusion every 3 weeks (intended dose intensity 45 mg/m²/week). If no dose-limiting toxicity was observed, patients were to receive 155 mg/m² and 175 mg/m² in subsequent courses. Hematopoietic growth factors were not to be used initially. In Study CA139-281 patients received TAXOL at 100 mg/m² as a 3-hour infusion every 2 weeks (intended dose intensity 50 mg/m²/week). In this study patients could be receiving hematopoietic growth factors before the start of TAXOL therapy, or this support was to be initiated as indicated; the dose of TAXOL was not increased. The dose intensity of TAXOL used in this patient population was lower than the dose intensity recommended for other solid tumors.

All patients had widespread and poor-risk disease. Applying the ACTG staging criteria to patients with prior systemic therapy, 93% were poor risk for extent of disease (T_1), 88% had a CD4 count <200 cells/mm³ (I_1), and 97% had poor risk considering their systemic illness (S_1).

All patients in Study CA139-174 had a Karnofsky performance status of 80 or 90 at baseline; in Study CA139-281, there were 26 (46%) patients with a Karnofsky performance status of 70 or worse at baseline.

Table 7: Extent of Disease at Study Entry

	Percent of Patients Prior Systemic Therapy (n=59)
Visceral ± edema ± oral ± cutaneous	42
Edema or lymph nodes ± oral ± cutaneous	41
Oral ± cutaneous	10
Cutaneous only	7

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DOXIL® is a registered trademark of ALZA Corporation.

Although the planned dose intensity in the two studies was slightly different (45 mg/m²/week in Study CA139-174 and 50 mg/m²/week in Study CA139-281), delivered dose intensity was 38-39 mg/m²/week in both studies, with a similar range (20-24 to 51-61).

Efficacy - The efficacy of TAXOL (paclitaxel) Injection was evaluated by assessing cutaneous tumor response according to the amended ACTG criteria and by seeking evidence of clinical benefit in patients in six domains of symptoms and/or conditions that are commonly related to AIDS-related Kaposi's sarcoma.

Cutaneous Tumor Response (Amended ACTG Criteria) - The objective response rate was 59% (95% CI: 46% to 72%)(35 of 59 patients) in patients with prior systemic therapy. Cutaneous responses were primarily defined as flattening of more than 50% of previously raised lesions.

Table 8: Overall Best Response (Amended ACTG Criteria)

	Percent of Patients Prior Systemic Therapy (n=59)
Complete response	3
Partial response	56
Stable disease	29
Progression	8
Early death/toxicity	3

The median time to response was 8.1 weeks and the median duration of response measured from the first day of treatment was 10.4 months (95% CI: 7.0 to 11.0 months) for the patients who had previously received systemic therapy. The median time to progression was 6.2 months (95% CI: 4.6 to 8.7 months).

Additional Clinical Benefit - Most data on patient benefit were assessed retrospectively (plans for such analyses were not included in the study protocols). Nonetheless, clinical descriptions and photographs indicated clear benefit in some patients, including instances of improved pulmonary function in patients with pulmonary involvement, improved ambulation, resolution of ulcers, and decreased analgesic requirements in patients with KS involving the feet and resolution of facial lesions and edema in patients with KS involving the face, extremities, and genitalia.

Safety - The adverse event profile of TAXOL administered to patients with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma was generally similar to that seen in the pooled analysis of data from 812 patients with solid tumors. These adverse events and adverse events from the Phase 2 second-line Kaposi's sarcoma studies are described in the **ADVERSE REACTIONS** section in tabular (Tables 10 and 16) and narrative form. In this immunosuppressed patient population, however, a lower dose intensity of TAXOL and supportive therapy including hematopoietic growth factors in patients with severe neutropenia are recommended. Patients with AIDS-related Kaposi's sarcoma may have more severe hematologic toxicities than patients with solid tumors.

INDICATIONS

TAXOL is indicated as first-line and subsequent therapy for the treatment of advanced carcinoma of the ovary. As first-line therapy, TAXOL is indicated in combination with cisplatin.

TAXOL is indicated for the adjuvant treatment of node-positive breast cancer administered sequentially to standard doxorubicin-containing combination chemotherapy. In the clinical trial, there was an overall favorable effect on disease-free and overall survival in the total population of patients with receptor-positive and receptor-negative tumors, but the benefit has been specifically demonstrated by available data (median follow-up 30 months) only in the patients with estrogen and progesterone receptor-negative tumors. (See **CLINICAL STUDIES: Breast Carcinoma.**)

TAXOL is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

TAXOL, in combination with cisplatin, is indicated for the first-line treatment of non-small cell lung cancer in patients who are not candidates for potentially curative surgery and/or radiation therapy.

TAXOL is indicated for the second-line treatment of AIDS-related Kaposi's sarcoma.

CONTRAINDICATIONS

TAXOL is contraindicated in patients who have a history of hypersensitivity reactions to TAXOL or other drugs formulated in Cremophor® EL (polyoxyethylated castor oil).

TAXOL should not be used in patients with solid tumors who have baseline neutrophil counts of < 1500 cells/mm³ or in patients with AIDS-related Kaposi's sarcoma with baseline neutrophil counts of <1000 cells/mm³.

WARNINGS

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2%-4% of patients receiving TAXOL in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H_2 antagonists. (See **DOSAGE AND ADMINISTRATION.**) Patients who experience severe hypersensitivity reactions to TAXOL should not be rechallenged with the drug.

Bone marrow suppression (primarily neutropenia) is dose-dependent and is the dose-limiting toxicity. Neutrophil nadirs occurred at a median of 11 days. TAXOL should not be administered to patients with baseline neutrophil counts of less than 1500 cells/mm³ (<1000 cells/mm³ for patients with KS). Frequent monitoring of blood counts should be instituted during TAXOL treatment. Patients should not be re-treated with subsequent cycles of TAXOL until neutrophils recover to a level >1500 cells/mm³ (>1000 cells/mm³ for patients with KS) and platelets recover to a level >100,000 cells/mm³.

Severe conduction abnormalities have been documented in < 1% of patients during TAXOL therapy and in some cases requiring pacemaker placement. If patients develop significant conduction abnormalities during TAXOL infusion, appropriate therapy should be administered and continuous cardiac monitoring should be performed during subsequent therapy with TAXOL.

Pregnancy: TAXOL can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel during the period of organogenesis to rabbits at doses of 3.0 mg/kg/day (about 0.2 the daily maximum recommended human dose on a mg/m² basis) caused embryo- and fetotoxicity, as indicated by intrauterine mortality, increased resorptions, and increased fetal deaths. Maternal toxicity was also observed at this dose. No teratogenic effects were observed at 1.0 mg/kg/day (about 1/15 the daily maximum recommended human dose on a mg/m² basis); teratogenic potential could not be assessed at higher doses due to extensive fetal mortality.

There are no adequate and well-controlled studies in pregnant women. If TAXOL (paclitaxel) Injection is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

PRECAUTIONS

Contact of the undiluted concentrate with plasticized polyvinyl chloride (PVC) equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted TAXOL solutions should preferably be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

TAXOL should be administered through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® filters which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP.

Drug Interactions: In a Phase I trial using escalating doses of TAXOL (110-200 mg/m²) and cisplatin (50 or 75 mg/m²) given as sequential infusions, myelosuppression was more profound when TAXOL was given after cisplatin than with the alternate sequence (ie, TAXOL before cisplatin). Pharmacokinetic data from these patients demonstrated a decrease in paclitaxel clearance of approximately 33% when TAXOL was administered following cisplatin.

The metabolism of TAXOL is catalyzed by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. In the absence of formal clinical drug interaction studies, caution should be exercised when administering TAXOL concomitantly with known substrates or inhibitors of the cytochrome P450 isoenzymes CYP2C8 and CYP3A4. (See **CLINICAL PHARMACOLOGY.**)

Potential interactions between TAXOL, a substrate of CYP3A4, and protease inhibitors (ritonavir, saquinavir, indinavir, and nelfinavir), which are substrates and/or inhibitors of CYP3A4, have not been evaluated in clinical trials.

Reports in the literature suggest that plasma levels of doxorubicin (and its active metabolite doxorubicinol) may be increased when paclitaxel and doxorubicin are used in combination.

Hematology: TAXOL therapy should not be administered to patients with baseline neutrophil counts of less than 1,500 cells/mm³. In order to monitor the occurrence of myelotoxicity, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving TAXOL. Patients should not be re-treated with subsequent cycles of TAXOL until neutrophils recover to a level >1500 cells/mm³ and platelets recover to a level >100,000 cells/mm³. In the case of severe neutropenia (<500 cells/mm³ for seven days or more) during a course of TAXOL therapy, a 20% reduction in dose for subsequent courses of therapy is recommended.

For patients with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma, TAXOL, at the recommended dose for this disease, can be initiated and repeated if the neutrophil count is at least 1000 cells/mm³.

Hypersensitivity Reactions: Patients with a history of severe hypersensitivity reactions to products containing Cremophor® EL (eg, cyclosporin for injection concentrate and teniposide for injection concentrate) should not be treated with TAXOL. In order to avoid the occurrence of severe hypersensitivity reactions, all patients treated with TAXOL should be premedicated with corticosteroids (such as dexamethasone), diphenhydramine and H_2 antagonists (such as cimetidine or ranitidine). Minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia do not require interruption of therapy. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of TAXOL and aggressive symptomatic therapy. Patients who have developed severe hypersensitivity reactions should not be rechallenged with TAXOL.

Cardiovascular: Hypotension, bradycardia, and hypertension have been observed during administration of TAXOL, but generally do not require treatment. Occasionally TAXOL infusions must be interrupted or discontinued because of initial or recurrent hypertension. Frequent vital sign monitoring, particularly during the first hour of TAXOL infusion, is recommended. Continuous cardiac monitoring is not required except for patients with serious conduction abnormalities. (See **WARNINGS.**)

Nervous System: Although the occurrence of peripheral neuropathy is frequent, the development of severe symptomatology is unusual and requires a dose reduction of 20% for all subsequent courses of TAXOL.

TAXOL contains dehydrated alcohol USP, 396 mg/mL; consideration should be given to possible CNS and other effects of alcohol. (See **PRECAUTIONS: Pediatric Use.**)

Hepatic: There is limited evidence that the myelotoxicity of TAXOL may be exacerbated in patients with serum total bilirubin >2 times ULN (see **CLINICAL PHARMACOLOGY**). Extreme caution should be exercised when administering TAXOL to such patients, with dose reduction as recommended in **DOSAGE AND ADMINISTRATION**, Table 17.

Injection Site Reaction: Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of TAXOL at a different site, ie, "recall", has been reported rarely.

Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been received as part of the continuing surveillance of TAXOL safety. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to ten days.

A specific treatment for extravasation reactions is unknown at this time. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.

Carcinogenesis, Mutagenesis, Impairment of Fertility: The carcinogenic potential of TAXOL has not been studied.

Paclitaxel has been shown to be clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). Paclitaxel was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.

Administration of paclitaxel prior to and during mating produced impairment of fertility in male and female rats at doses equal to or greater than 1 mg/kg/day (about 0.04 the daily maximum recommended human dose on a mg/m² basis). At this dose, paclitaxel caused reduced fertility and reproductive indices, and increased embryo- and fetotoxicity. (See **WARNINGS.**)

Pregnancy: Pregnancy "Category D". (See **WARNINGS.**)

Nursing Mothers: It is not known whether the drug is excreted in human milk. Following intravenous administration of carbon-14 labeled TAXOL (paclitaxel) Injection to rats on days 9 to 10 postpartum, concentrations of radioactivity in milk were higher than in plasma and declined in parallel with the plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving TAXOL therapy.

Pediatric Use: The safety and effectiveness of TAXOL in pediatric patients have not been established.

There have been reports of central nervous system (CNS) toxicity (rarely associated with death) in a clinical trial in pediatric patients in which TAXOL was infused intravenously over 3 hours at doses ranging from 350 mg/m² to 420 mg/m². The toxicity is most likely attributable to the high dose of the ethanol component of the TAXOL vehicle given over a short infusion time. The use of concomitant antihistamines may intensify this effect. Although a direct effect of the paclitaxel itself cannot be discounted, the high doses used in this study (over twice the recommended adult dosage) must be considered in assessing the safety of TAXOL for use in this population.

Geriatric Use: Of 2228 patients who received TAXOL in eight clinical studies evaluating its safety and effectiveness in the treatment of advanced ovarian cancer, breast carcinoma, or NSCLC, and 1570 patients who were randomized to receive TAXOL in the adjuvant breast cancer study, 649 patients (17%) were 65 years or older and 49 patients (1%) were 75 years or older. In most studies, severe myelosuppression was more frequent in elderly patients; in some studies, severe neuropathy was more common in elderly patients. In two clinical studies in NSCLC, the elderly patients treated with TAXOL had a higher incidence of cardiovascular events. Estimates of efficacy appeared similar in elderly patients and in younger patients; however, comparative efficacy cannot be determined with confidence due to the small number of elderly patients studied. In a study of first-line treatment of ovarian cancer, elderly patients had a lower median survival than younger patients, but no other efficacy parameters favored the younger group. Table 9 presents the incidences of Grade IV neutropenia and severe neuropathy in clinical studies according to age.

Table 9: Selected Adverse Events in Geriatric Patients Receiving TAXOL in Clinical Studies

	Patients [n/total (%)]			
	Neutropenia (Grade IV)		Peripheral Neuropathy (Grades III/IV)	
INDICATION	Age (yrs)		Age (yrs)	
(Study/Regimen)	≥65	<65	≥65	<65
• **OVARIAN Cancer**				
(Intergroup First-Line/ T175/3 c75[a])	34/83 (41)	78/252 (31)	24/84 (29)*[b]	46/255 (18)[b]
(GOG-111 First-Line/ T135/24 c75[a])	48/61 (79)	106/129 (82)	3/62 (5)	2/134 (1)
(Phase 3 Second-Line/ T175/3[c])	5/19 (26)	21/76 (28)	1/19 (5)	0/76 (0)
(Phase 3 Second-Line/ T175/24[c])	21/25 (84)	57/79 (72)	0/25 (0)	2/80 (3)
(Phase 3 Second-Line/ T135/3[c])	4/16 (25)	10/81 (12)	0/17 (0)	0/81 (0)
(Phase 3 Second-Line/ T135/24[c])	17/22 (77)	53/83 (64)	0/22 (0)	0/83 (0)
(Phase 3 Second-Line Pooled)	47/82 (57)*	141/319 (44)	1/83 (1)	2/320 (1)
• **Adjuvant BREAST Cancer**				
(Intergroup/AC followed by T[d])	56/102 (55)	734/1468 (50)	5/102 (5)[e]	46/1468 (3)[e]
• **BREAST Cancer After Failure of Initial Therapy**				
(Phase 3/T175/3[c])	7/24 (29)	56/200 (28)	3/25 (12)	12/204 (6)
(Phase 3/T135/3[c])	7/20 (35)	37/207 (18)	0/20 (0)	6/209 (3)
• **Non-Small Cell LUNG Cancer**				
(ECOG/T135/24 c75[a])	58/71 (82)	86/124 (69)	9/71 (13)[f]	16/124 (13)[f]
(Phase 3/T175/3 c80[a])	37/89 (42)*	56/267 (21)	11/91 (12)*	11/271 (4)

* $p<0.05$

[a] TAXOL dose in mg/m²/infusion duration in hours; cisplatin doses in mg/m².

[b] Peripheral neuropathy was included within the neurotoxicity category in the Intergroup First-Line Ovarian Cancer study (see Table 11).

[c] TAXOL dose in mg/m²/infusion duration in hours.

[d] TAXOL (T) following four courses of doxorubicin and cyclophosphamide (AC) at a dose of 175 mg/m²/3 hours every 3 weeks for four courses.

[e] Peripheral neuropathy reported as neurosensory toxicity in the Intergroup Adjuvant Breast Cancer study (see Table 13).

[f] Peripheral neuropathy reported as neurosensory toxicity in the ECOG NSCLC study (see Table 15).

Information for Patients: (See **Patient Information Leaflet.**)

ADVERSE REACTIONS

Pooled Analysis of Adverse Event Experiences from Single-Agent Studies: Data in the following table are based on the experience of 812 patients (493 with ovarian carcinoma and 319 with breast carcinoma) enrolled in 10 studies who received single-agent TAXOL. Two hundred and seventy-five patients were treated in eight Phase 2 studies with TAXOL doses ranging from 135 to 300 mg/m² administered over 24 hours (in four of these studies, G-CSF was administered as hematopoietic support). Three hundred and one patients were treated in the randomized Phase 3 ovarian carcinoma study which compared two doses (135 or 175 mg/m²) and two schedules (3 or 24 hours) of TAXOL. Two hundred and thirty-six patients with breast carcinoma received TAXOL (135 or 175 mg/m²) administered over 3 hours in a controlled study.

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Table 10: Summary[a] of Adverse Events in Patients With Solid Tumors Receiving Single-Agent TAXOL (paclitaxel) Injection

		Percent of Patients (n=812)
• **Bone Marrow**		
- Neutropenia	< 2,000/mm³	90
	< 500/mm³	52
- Leukopenia	< 4,000/mm³	90
	< 1,000/mm³	17
- Thrombocytopenia	< 100,000/mm³	20
	< 50,000/mm³	7
- Anemia	< 11 g/dL	78
	< 8 g/dL	16
- Infections		30
- Bleeding		14
- Red Cell Transfusions		25
- Platelet Transfusions		2
• **Hypersensitivity Reaction**[b]		
- All		41
- Severe[†]		2
• **Cardiovascular**		
- Vital Sign Changes[c]		
- Bradycardia (n=537)		3
- Hypotension (n=532)		12
- Significant Cardiovascular Events		1
• **Abnormal ECG**		
- All Pts		23
- Pts with normal baseline (n=559)		14
• **Peripheral Neuropathy**		
- Any symptoms		60
- Severe symptoms[†]		3
• **Myalgia/Arthralgia**		
- Any symptoms		60
- Severe symptoms[†]		8
• **Gastrointestinal**		
- Nausea and vomiting		52
- Diarrhea		38
- Mucositis		31
• **Alopecia**		87
• **Hepatic** (Pts with normal baseline and on study data)		
- Bilirubin elevations (N=765)		7
- Alkaline phosphatase elevations (N=575)		22
- AST (SGOT) elevations (N=591)		19
• **Injection Site Reaction**		13

[a] Based on worst course analysis.
[b] All patients received premedication.
[c] During the first 3 hours of infusion.
[†] Severe events are defined as at least Grade III toxicity.

None of the observed toxicities were clearly influenced by age.

Disease-Specific Adverse Event Experiences First-Line Ovary in Combination: For the 1084 patients who were evaluable for safety in the Phase 3 first-line ovary combination therapy studies, Table 11 shows the incidence of important adverse events. For both studies, the analysis of safety was based on all courses of therapy (six courses for the GOG-111 study and up to nine courses for the Intergroup study).

Table 11: Frequency[a] of Important Adverse Events in the Phase 3 First-Line Ovarian Carcinoma Studies

	Percent of Patients			
	Intergroup		GOG-111	
	T175/3[b] c75[c] (n=339)	C750[c] c75[c] (n=336)	T135/24[b] c75[c] (n=196)	C750[c] c75[c] (n=213)
• **Bone Marrow**				
- Neutropenia				
< 2000/mm³	91[d]	95[d]	96	92
< 500/mm³	33[d]	43[d]	81[d]	58[d]
- Thrombocytopenia				
< 100,000/mm³[e]	21[d]	33[d]	26	30
< 50,000/mm³	3[d]	7[d]	10	9
- Anemia				
< 11 g/dL[f]	96	97	88	86
< 8 g/dL	3[d]	8[d]	13	9
- Infections	25	27	21	15
- Febrile Neutropenia	4	7	15[d]	4[d]
• **Hypersensitivity Reaction**				
- All	11[d]	6[d]	8[d,g]	1[d,g]
- Severe[†]	1	1	3[d,g]	---[d,g]
• **Neurotoxicity**[h]				
- Any symptoms	87[d]	52[d]	25	20
- Severe symptoms[†]	21[d]	2[d]	3[d]	---[d]
• **Nausea and Vomiting**				
- Any symptoms	88	93	65	69
- Severe symptoms[†]	18	24	10	11
• **Myalgia/Arthralgia**				
- Any symptoms	60[d]	27[d]	9[d]	2[d]
- Severe symptoms[†]	6[d]	1[d]	1	---
• **Diarrhea**				
- Any symptoms	37[d]	29[d]	16[d]	8[d]
- Severe symptoms[†]	2	3	4	1
• **Asthenia**				
- Any symptoms	NC	NC	17[d]	10[d]
- Severe symptoms[†]	NC	NC	1	1
• **Alopecia**				
- Any symptoms	96[d]	89[d]	55[d]	37[d]
- Severe symptoms[†]	51[d]	21[d]	6	8

[a] Based on worst course analysis.
[b] TAXOL (T) dose in mg/m²/infusion duration in hours.
[c] Cyclophosphamide (C) or cisplatin (c) dose in mg/m².
[d] p<0.05 by Fisher exact test.
[e] <130,000/mm³ in the Intergroup study.
[f] <12 g/dL in the Intergroup study.
[g] All patients received premedication.
[h] In the GOG-111 study, neurotoxicity was collected as peripheral neuropathy and in the Intergroup study, neurotoxicity was collected as either neuromotor or neurosensory symptoms.
[†] Severe events are defined as at least Grade III toxicity.
NC Not Collected.

Second-Line Ovary: For the 403 patients who received single-agent TAXOL (paclitaxel) Injection in the Phase 3 second-line ovarian carcinoma study, the following table shows the incidence of important adverse events.

Table 12: Frequency[a] of Important Adverse Events in the Phase 3 Second-Line Ovarian Carcinoma Study

		Percent of Patients			
		175/3[b] (n=95)	175/24[b] (n=105)	135/3[b] (n=98)	135/24[b] (n=105)
• **Bone Marrow**					
- Neutropenia	< 2,000/mm³	78	98	78	98
	< 500/mm³	27	75	14	67
- Thrombocytopenia	< 100,000/mm³	4	18	8	6
	< 50,000/mm³	1	7	2	1
- Anemia	< 11 g/dL	84	90	68	88
	< 8 g/dL	11	12	6	10
- Infections		26	29	20	18
• **Hypersensitivity Reaction**[c]					
- All		41	45	38	45
- Severe[†]		2	0	2	1
• **Peripheral Neuropathy**					
- Any symptoms		63	60	55	42
- Severe symptoms[†]		1	2	0	0
• **Mucositis**					
- Any symptoms		17	35	21	25
- Severe symptoms[†]		0	3	0	2

[a] Based on worst course analysis.
[b] TAXOL dose in mg/m²/infusion duration in hours.
[c] All patients received premedication.
[†] Severe events are defined as at least Grade III toxicity.

Myelosuppression was dose and schedule related, with the schedule effect being more prominent. The development of severe hypersensitivity reactions (HSRs) was rare; 1% of the patients and 0.2% of the courses overall. There was no apparent dose or schedule effect seen for the HSRs. Peripheral neuropathy was clearly dose-related, but schedule did not appear to affect the incidence.

Adjuvant Breast: For the Phase 3 adjuvant breast carcinoma study, the following table shows the incidence of important severe adverse events for the 3121 patients (total population) who were evaluable for safety as well as for a group of 325 patients (early population) who, per the study protocol, were monitored more intensively than other patients.

Table 13: Frequency[a] of Important Severe[b] Adverse Events in the Phase 3 Adjuvant Breast Carcinoma Study

	Percent of Patients			
	Early Population		Total Population	
	AC[c] (n=166)	AC[c] followed by T[d] (n=159)	AC[c] (n=1551)	AC[c] followed by T[d] (n=1570)
• **Bone Marrow**[e]				
- Neutropenia				
< 500/mm³	79	76	48	50
- Thrombocytopenia				
< 50,000/mm³	27	25	11	11
- Anemia				
< 8 g/dL	17	21	8	8
- Infections	6	14	5	6
- Fever without Infection	–	3	<1	1
• **Hypersensitivity Reaction**[f]	1	4	1	2
• **Cardiovascular Events**	1	2	1	2
• **Neuromotor Toxicity**	1	1	<1	1
• **Neurosensory Toxicity**	–	3	<1	3
• **Myalgia/Arthralgia**	–	2	<1	2
• **Nausea/Vomiting**	13	18	8	9
• **Mucositis**	13	4	6	5

[a] Based on worst course analysis.
[b] Severe events are defined as at least Grade III toxicity.
[c] Patients received 600 mg/m² cyclophosphamide and doxorubicin (AC) at doses of either 60 mg/m², 75 mg/m², or 90 mg/m² (with prophylactic G-CSF support and ciprofloxacin), every 3 weeks for four courses.
[d] TAXOL (T) following four courses of AC at a dose of 175 mg/m²/3 hours every 3 weeks for four courses.
[e] The incidence of febrile neutropenia was not reported in this study.
[f] All patients were to receive premedication.

The incidence of an adverse event for the total population likely represents an underestimation of the actual incidence given that safety data were collected differently based on enrollment cohort. However, since safety data were collected consistently across regimens, the safety of the sequential addition of TAXOL following AC therapy may be compared with AC therapy alone. Compared to patients who received AC alone, patients who received AC followed by TAXOL experienced more Grade III/IV neurosensory toxicity, more Grade III/IV myalgia/arthralgia, more Grade III/IV neurologic pain (5% vs 1%), more Grade III/IV flu-like symptoms (5% vs 3%), and more Grade III/IV hyperglycemia (3% vs 1%). During the additional four courses of treatment with TAXOL, two deaths (0.1%) were attributed to treatment. During TAXOL treatment, Grade IV neutropenia was reported for 15% of patients, Grade II/III neurosensory toxicity for 15%, Grade II/III myalgias for 23%, and alopecia for 46%.

The incidences of severe hematologic toxicities, infections, mucositis, and cardiovascular events increased with higher doses of doxorubicin.

Breast Cancer After Failure of Initial Chemotherapy: For the 458 patients who received single-agent TAXOL in the Phase 3 breast carcinoma study, the following table shows the incidence of important adverse events by treatment arm (each arm was administered by a 3-hour infusion).

Table 14: Frequency[a] of Important Adverse Events in the Phase 3 Study of Breast Cancer After Failure of Initial Chemotherapy or Within 6 Months of Adjuvant Chemotherapy

		Percent of Patients	
		175/3[b] (n=229)	135/3[b] (n=229)
• **Bone Marrow**			
- Neutropenia	< 2,000/mm³	90	81
	< 500/mm³	28	19
- Thrombocytopenia	< 100,000/mm³	11	7
	< 50,000/mm³	3	2
- Anemia	< 11 g/dL	55	47
	< 8 g/dL	4	2
- Infections		23	15
- Febrile Neutropenia		2	2
• **Hypersensitivity Reaction**[c]			
- All		36	31
- Severe[†]		0	<1
• **Peripheral Neuropathy**			
- Any symptoms		70	46
- Severe symptoms[†]		7	3
• **Mucositis**			
- Any symptoms		23	17
- Severe symptoms[†]		3	<1

[a] Based on worst course analysis.
[b] TAXOL dose in mg/m²/infusion duration in hours.
[c] All patients received premedication.
[†] Severe events are defined as at least Grade III toxicity.

Myelosuppression and peripheral neuropathy were dose related. There was one severe hypersensitivity reaction (HSR) observed at the dose of 135 mg/m².

First-Line NSCLC in Combination: In the study conducted by the Eastern Cooperative Oncology Group (ECOG), patients were randomized to either TAXOL (paclitaxel) Injection (T) 135 mg/m² as a 24-hour infusion in combination with cisplatin (c) 75 mg/m², TAXOL (T) 250 mg/m² as a 24-hour infusion in combination with cisplatin (c) 75 mg/m² with G-CSF support, or cisplatin (c) 75 mg/m² on day 1, followed by etoposide (VP) 100 mg/m² on days 1, 2 and 3 (control).

The following table shows the incidence of important adverse events.

Table 15: Frequency[a] of Important Adverse Events in the Phase 3 Study for First-Line NSCLC

		Percent of Patients		
		T135/24[b] c75 (n=195)	T250/24[c] c75 (n=197)	VP100[d] c75 (n=196)
• **Bone Marrow**				
- Neutropenia	< 2,000/mm³	89	86	84
	< 500/mm³	74[e]	65	55
- Thrombocytopenia	< normal	48	68	62
	< 50,000/mm³	6	12	16
- Anemia	< normal	94	96	95
	< 8 g/dL	22	19	28
- Infections		38	31	35
• **Hypersensitivity Reaction**[f]				
- All		16	27	13
- Severe[†]		1	4[e]	1
• **Arthralgia/Myalgia**				
- Any symptoms		21[e]	42[e]	9
- Severe symptoms[†]		3	11	1
• **Nausea/Vomiting**				
- Any symptoms		85	87	81
- Severe symptoms[†]		27	29	22
• **Mucositis**				
- Any symptoms		18	28	16
- Severe symptoms[†]		1	4	2
• **Neuromotor Toxicity**				
- Any symptoms		37	47	44
- Severe symptoms[†]		6	12	7
• **Neurosensory Toxicity**				
- Any symptoms		48	61	25
- Severe symptoms[†]		13	28[e]	8
• **Cardiovascular Events**				
- Any symptoms		33	39	24
- Severe symptoms[†]		13	12	8

[a] Based on worst course analysis.
[b] TAXOL (T) dose in mg/m²/infusion duration in hours; cisplatin (c) dose in mg/m².
[c] TAXOL dose in mg/m²/infusion duration in hours with G-CSF support; cisplatin dose in mg/m².
[d] Etoposide (VP) dose in mg/m² was administered I.V. on days 1, 2 and 3; cisplatin dose in mg/m².
[e] p<0.05.
[f] All patients received premedication.
[†] Severe events are defined as at least Grade III toxicity.

Toxicity was generally more severe in the high-dose TAXOL (paclitaxel) Injection treatment arm (T250/c75) than in the low-dose TAXOL arm (T135/c75). Compared to the cisplatin/etoposide arm, patients in the low-dose TAXOL arm experienced more arthralgia/myalgia of any grade and more severe neutropenia. The incidence of febrile neutropenia was not reported in this study.

Kaposi's Sarcoma: The following table shows the frequency of important adverse events in the 85 patients with KS treated with two different single-agent TAXOL regimens.

Table 16: Frequency[a] of Important Adverse Events in the AIDS-Related Kaposi's Sarcoma Studies

		Percent of Patients	
		Study CA139-174 TAXOL 135/3[b] q 3 wk (n=29)	Study CA139-281 TAXOL 100/3[b] q 2 wk (n=56)
• **Bone Marrow**			
- Neutropenia	< 2,000/mm³	100	95
	< 500/mm³	76	35
- Thrombocytopenia	< 100,000/mm³	52	27
	< 50,000/mm³	17	5
- Anemia	< 11 g/dL	86	73
	< 8 g/dL	34	25
- Febrile Neutropenia		55	9
• **Opportunistic Infection**			
- Any		76	54
- Cytomegalovirus		45	27
- Herpes Simplex		38	11
- *Pneumocystis carinii*		14	21
- *M. avium-intracellulare*		24	4
- Candidiasis, esophageal		7	9
- Cryptosporidiosis		7	7
- Cryptococcal meningitis		3	2
- Leukoencephalopathy		–	2
• **Hypersensitivity Reaction[c]**			
- All		14	9
• **Cardiovascular**			
- Hypotension		17	9
- Bradycardia		3	–
• **Peripheral Neuropathy**			
- Any		79	46
- Severe[†]		10	2
• **Myalgia/Arthralgia**			
- Any		93	48
- Severe[†]		14	16
• **Gastrointestinal**			
- Nausea and Vomiting		69	70
- Diarrhea		90	73
- Mucositis		45	20
• **Renal (creatinine elevation)**			
- Any		34	18
- Severe[†]		7	5
• **Discontinuation for drug toxicity**		7	16

[a] Based on worst course analysis.
[b] TAXOL dose in mg/m²/infusion duration in hours.
[c] All patients received premedication.
[†] Severe events are defined as at least Grade III toxicity.

As demonstrated in this table, toxicity was more pronounced in the study utilizing TAXOL (paclitaxel) injection at a dose of 135 mg/m² every 3 weeks than in the study utilizing TAXOL at a dose of 100 mg/m² every 2 weeks. Notably, severe neutropenia (76% vs. 35%), febrile neutropenia (55% vs. 9%), and opportunistic infections (76% vs. 54%) were more common with the former dose and schedule. The differences between the two studies with respect to dose escalation and use of hematopoietic growth factors, as described above, should be taken into account. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma.**) Note also that only 26% of the 85 patients in these studies received concomitant treatment with protease inhibitors, whose effect on paclitaxel metabolism has not yet been studied.

Adverse Event Experiences by Body System: Unless otherwise noted, the following discussion refers to the overall safety database of 812 patients with solid tumors treated with single-agent TAXOL in clinical studies. Toxicities that occurred with greater severity or frequency in previously untreated patients with ovarian carcinoma or NSCLC who received TAXOL in combination with cisplatin or in patients with breast cancer who received TAXOL after doxorubicin/cyclophosphamide in the adjuvant setting and that occurred with a difference that was clinically significant in these populations are also described. The frequency and severity of important adverse events for the Phase 3 ovarian carcinoma, breast carcinoma, NSCLC, and the Phase 2 Kaposi's sarcoma studies are presented above in tabular form by treatment arm. In addition, rare events have been reported from postmarketing experience or from other clinical studies. The frequency and severity of adverse events have been generally similar for patients receiving TAXOL for the treatment of ovarian, breast, or lung carcinoma or Kaposi's sarcoma, but patients with AIDS-related Kaposi's sarcoma may have more frequent and severe hematologic toxicity, infections, and febrile neutropenia. These patients require a lower dose intensity and supportive care. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma.**) Toxicities that were observed only in or were noted to have occurred with greater severity in the population with Kaposi's sarcoma and that occurred with a difference that was clinically significant in this population are described.

Hematologic: Bone marrow suppression was the major dose-limiting toxicity of TAXOL. Neutropenia, the most important hematologic toxicity, was dose and schedule dependent and was generally rapidly reversible. Among patients treated in the Phase 3 second-line ovarian study with a 3-hour infusion, neutrophil counts declined below 500 cells/mm³ in 14% of the patients treated with a dose of 135 mg/m² compared to 27% at a dose of 175 mg/m² (p=0.05). In the same study, severe neutropenia (<500 cells/mm³) was more frequent with the 24-hour than with the 3-hour infusion; infusion duration had a greater impact on myelosuppression than dose. Neutropenia did not appear to increase with cumulative exposure and did not appear to be more frequent nor more severe for patients previously treated with radiation therapy.

In the study where TAXOL was administered to patients with ovarian carcinoma at a dose of 135 mg/m²/24 hours in combination with cisplatin versus the control arm of cyclosphosphamide plus cisplatin, the incidences of grade IV neutropenia and of febrile neutropenia were significantly greater in the TAXOL plus cisplatin arm than in the control arm. Grade IV neutropenia occurred in 81% on the TAXOL plus cisplatin arm versus 58% on the cyclophosphamide plus cisplatin arm, and febrile neutropenia occurred in 15% and 4% respectively. On the TAXOL/cisplatin arm, there were 35/1074 (3%) courses with fever in which Grade IV neutropenia was reported at some time during the course. When TAXOL followed by cisplatin was administered to patients with advanced NSCLC in the ECOG study, the incidences of Grade IV neutropenia were 74% (TAXOL 135 mg/m²/24 hours followed by cisplatin) and 65% (TAXOL 250 mg/m²/24 hours followed by cisplatin and G-CSF) compared with 55% in patients who received cisplatin/etoposide.

Fever was frequent (12% of all treatment courses). Infectious episodes occurred in 30% of all patients and 9% of all courses; these episodes were fatal in 1% of all patients, and included sepsis, pneumonia and peritonitis. In the Phase 3 second-line ovarian study, infectious episodes were reported in 20% and 26% of the patients treated with a dose of 135 mg/m² or 175 mg/m² given as 3-hour infusions, respectively. Urinary tract infections and upper respiratory tract infections were the most frequently reported infectious complications. In the immunosuppressed patient population with advanced HIV disease and poor-risk AIDS-related Kaposi's sarcoma, 61% of the patients reported at least one opportunistic infection. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma.**) The use of supportive therapy, including G-CSF, is recommended for patients who have experienced severe neutropenia. (See **DOSAGE AND ADMINISTRATION.**)

Thrombocytopenia was uncommon, and almost never severe (<50,000 cells/mm³). Twenty percent of the patients experienced a drop in their platelet count below 100,000 cells/mm³ at least once while on treatment; 7% had a platelet count <50,000 cells/mm³ at the time of their worst nadir. Bleeding episodes were reported in 4% of all courses and by 14% of all patients but most of the hemorrhagic episodes were localized and the frequency of these events was unrelated to the TAXOL (paclitaxel) injection dose and schedule. In the Phase 3 second-line ovarian study, bleeding episodes were reported in 10% of the patients; no patients treated with the 3-hour infusion received platelet transfusions. In the adjuvant breast carcinoma trial, the incidence of severe thrombocytopenia and platelet transfusions increased with higher doses of doxorubicin.

Anemia (Hb <11 g/dL) was observed in 78% of all patients and was severe (Hb <8 g/dL) in 16% of the cases. No consistent relationship between dose or schedule and the frequency of anemia was observed. Among all patients with normal baseline hemoglobin, 69% became anemic on study but only 7% had severe anemia. Red cell transfusions were required in 25% of all patients and in 12% of those with normal baseline hemoglobin levels.

Hypersensitivity Reactions (HSRs): All patients received premedication prior to TAXOL (see **WARNINGS** and **PRECAUTIONS: Hypersensitivity Reactions**). The frequency and severity of HSRs were not affected by the dose or schedule of TAXOL administration. In the Phase 3 second-line ovarian study, the 3-hour infusion was not associated with a greater increase in HSRs when compared to the 24-hour infusion. Hypersensitivity reactions were observed in 20% of all courses and in 41% of all patients. These reactions were severe in less than 2% of the patients and 1% of the courses. No severe reactions were observed after course 3 and severe symptoms occurred generally within the first hour of TAXOL infusion. The most frequent symptoms observed during these severe reactions were dyspnea, flushing, chest pain, and tachycardia.

The minor hypersensitivity reactions consisted mostly of flushing (28%), rash (12%), hypotension (4%), dyspnea (2%), tachycardia (2%), and hypertension (1%). The frequency of hypersensitivity reactions remained relatively stable during the entire treatment period.

Rare reports of chills and reports of back pain in association with hypersensitivity reactions have been received as part of the continuing surveillance of TAXOL safety.

Cardiovascular: Hypotension, during the first 3 hours of infusion, occurred in 12% of all patients and 3% of all courses administered. Bradycardia, during the first 3 hours of infusion, occurred in 3% of all patients and 1% of all courses. In the Phase 3 second-line ovarian study, neither dose nor schedule had an effect on the frequency of hypotension and bradycardia. These vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation. The frequency of hypotension and bradycardia were not influenced by prior anthracycline therapy.

Significant cardiovascular events possibly related to single-agent TAXOL occurred in approximately 1% of all patients. These events included syncope, rhythm abnormalities, hypertension and venous thrombosis. One of the patients with syncope treated with TAXOL at 175 mg/m² over 24 hours had progressive hypotension and died. The arrhythmias included asymptomatic ventricular tachycardia, bigeminy and complete AV block requiring pacemaker placement. Among patients with NSCLC treated with TAXOL in combination with cisplatin in the Phase 3 study, significant cardiovascular events occurred in 12%-13%. This apparent increase in cardiovascular events is possibly due to an increase in cardiovascular risk factors in patients with lung cancer.

Electrocardiogram (ECG) abnormalities were common among patients at baseline. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. ECG abnormalities were noted in 23% of all patients. Among patients with a normal ECG prior to study entry, 14% of all patients developed an abnormal tracing while on study. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, sinus tachycardia, and premature beats. Among patients with normal ECGs at baseline, prior therapy with anthracyclines did not influence the frequency of ECG abnormalities.

Cases of myocardial infarction have been reported rarely. Congestive heart failure has been reported typically in patients who have received other chemotherapy, notably anthracyclines. (See **PRECAUTIONS: Drug Interactions.**)

Rare reports of atrial fibrillation and supraventricular tachycardia have been received as part of the continuing surveillance of TAXOL safety.

Respiratory: Rare reports of interstitial pneumonia, lung fibrosis, and pulmonary embolism have been received as part of the continuing surveillance of TAXOL safety. Rare reports of radiation pneumonitis have been received in patients receiving concurrent radiotherapy.

Neurologic: The assessment of neurologic toxicity was conducted differently among the studies as evident from the data reported in each individual study (see Tables 10-16). Moreover, the frequency and severity of neurologic manifestations were influenced by prior and/or concomitant therapy with neurotoxic agents.

In general, the frequency and severity of neurologic manifestations were dose-dependent in patients receiving single-agent TAXOL. Peripheral neuropathy was observed in 60% of all patients (3% severe) and in 52% (2% severe) of the patients without pre-existing neuropathy. The frequency of peripheral neuropathy increased with cumulative dose. Neurologic symptoms were observed in 27% of the patients after the first course of treatment and in 34%-51% from course 2 to 10. Peripheral neuropathy was the cause of TAXOL discontinuation in 1% of all patients. Sensory symptoms have usually improved or resolved within several months of TAXOL discontinuation. Pre-existing neuropathies resulting from prior therapies are not a contraindication for TAXOL therapy.

In the Intergroup first-line ovarian carcinoma study (see Table 11), neurotoxicity included reports of neuromotor and neurosensory events. The regimen with TAXOL 175 mg/m² given by 3-hour infusion plus cisplatin 75 mg/m² resulted in a greater incidence and severity of neurotoxicity than the regimen containing cyclophosphamide and cisplatin, 87% (21% severe) versus 52% (2% severe), respectively. The duration of grade III or IV neurotoxicity cannot be determined with precision for the Intergroup study since the resolution dates of adverse events were not collected in the case report forms for this trial and complete follow-up documentation was available only in a minority of these patients. In the GOG first-line ovarian carcinoma study, neurotoxicity was reported as peripheral neuropathy. The regimen with TAXOL (paclitaxel) injection 135 mg/m² given by 24-hour infusion plus cisplatin 75 mg/m² resulted in an incidence of neurotoxicity that was similar to the regimen containing cyclophosphamide plus cisplatin, 25% (3% severe) versus 20% (0% severe), respectively. Cross-study comparison of neurotoxicity in the Intergroup and GOG trials suggests that when TAXOL is given in combination with cisplatin 75 mg/m², the incidence of severe neurotoxicity is more common at a TAXOL dose of 175 mg/m² given by 3-hour infusion (21%) than at a dose of 135 mg/m² given by 24-hour infusion (3%).

In patients with NSCLC, administration of TAXOL followed by cisplatin resulted in a greater incidence of severe neurotoxicity compared to the incidence in patients with ovarian or breast cancer treated with single-agent TAXOL (paclitaxel) injection. Severe neurosensory symptoms were noted in 13% of NSCLC patients receiving TAXOL 135 mg/m² by 24-hour infusion followed by cisplatin 75 mg/m² and 8% of NSCLC patients receiving cisplatin/etoposide (see Table 15).

Other than peripheral neuropathy, serious neurologic events following TAXOL administration have been rare (<1%) and have included grand mal seizures, syncope, ataxia, and neuroencephalopathy.

Rare reports of autonomic neuropathy resulting in paralytic ileus have been received as part of the continuing surveillance of TAXOL safety. Optic nerve and/or visual disturbances (scintillating scotomata) have also been reported, particularly in patients who have received higher doses than those recommended. These effects generally have been reversible. However, rare reports in the literature of abnormal visual evoked potentials in patients have suggested persistent optic nerve damage. Postmarketing reports of ototoxicity (hearing loss and tinnitus) have also been received.

Arthralgia/Myalgia: There was no consistent relationship between dose or schedule of TAXOL and the frequency or severity of arthralgia/myalgia. Sixty percent of all patients treated experienced arthralgia/myalgia; 8% experienced severe symptoms. The symptoms were usually transient, occurred two or three days after TAXOL administration, and resolved within a few days. The frequency and severity of musculoskeletal symptoms remained unchanged throughout the treatment period.

Hepatic: No relationship was observed between liver function abnormalities and either dose or schedule of TAXOL administration. Among patients with normal baseline liver function 7%, 22%, and 19% had elevations in bilirubin, alkaline phosphatase, and AST (SGOT), respectively. Prolonged exposure to TAXOL was not associated with cumulative hepatic toxicity.

Rare reports of hepatic necrosis and hepatic encephalopathy leading to death have been received as part of the continuing surveillance of TAXOL safety.

Renal: Among the patients treated for Kaposi's sarcoma with TAXOL, five patients had renal toxicity of grade III or IV severity. One patient with suspected HIV nephropathy of grade IV severity had to discontinue therapy. The other four patients had renal insufficiency with reversible elevations of serum creatinine.

Gastrointestinal (GI): Nausea/vomiting, diarrhea, and mucositis were reported by 52%, 38%, and 31% of all patients, respectively. These manifestations were usually mild to moderate. Mucositis was schedule dependent and occurred more frequently with the 24-hour than with the 3-hour infusion.

In patients with poor-risk AIDS-related Kaposi's sarcoma, nausea/vomiting, diarrhea, and mucositis were reported by 69%, 79%, and 28% of patients, respectively. One third of patients with Kaposi's sarcoma complained of diarrhea prior to study start. (See **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma.**)

In the first-line Phase 3 ovarian carcinoma studies, the incidence of nausea and vomiting when TAXOL was administered in combination with cisplatin appeared to be greater compared with the database for single-agent TAXOL in ovarian and breast carcinoma. In addition, diarrhea of any grade was reported more frequently compared to the control arm, but there was no difference for severe diarrhea in these studies.

Rare reports of intestinal obstruction, intestinal perforation, pancreatitis, ischemic colitis, and dehydration have been received as part of the continuing surveillance of TAXOL safety. Rare reports of neutropenic enterocolitis (typhlitis), despite the coadministration of G-CSF, were observed in patients treated with TAXOL alone and in combination with other chemotherapeutic agents.

Injection Site Reaction: Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of TAXOL at a different site, ie, "recall", has been reported rarely.

Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been received as part of the continuing surveillance of TAXOL safety. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to ten days.

A specific treatment for extravasation reactions is unknown at this time. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration.

Other Clinical Events: Alopecia was observed in almost all (87%) of the patients. Transient skin changes due to TAXOL-related hypersensitivity reactions have been observed, but no other skin toxicities were significantly associated with TAXOL administration. Nail changes (changes in pigmentation or discoloration of nail bed) were uncommon (2%). Edema was reported in 21% of all patients (17% of those without baseline edema); only 1% had severe edema and none of these patients required treatment discontinuation. Edema was most commonly focal and disease-related. Edema was observed in 5% of all courses for patients with normal baseline and did not increase with time on study.

Rare reports of skin abnormalities related to radiation recall as well as reports of maculopapular rash, pruritus, Stevens-Johnson syndrome, and toxic epidermal necrolysis have been received as part of the continuing surveillance of TAXOL safety.

Reports of asthenia and malaise have been received as part of the continuing surveillance of TAXOL safety. In the Phase 3 trial of TAXOL 135 mg/m² over 24 hours in combination with cisplatin as first-line therapy of ovarian cancer, asthenia was reported in 17% of the patients, significantly greater than the 10% incidence observed in the control arm of cyclophosphamide/cisplatin.

Rare reports of conjunctivitis and increased lacrimation have been received as part of the continuing surveillance of TAXOL safety.

Accidental Exposure: Upon inhalation, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported. Following topical exposure, events have included tingling, burning, and redness.

OVERDOSAGE

There is no known antidote for TAXOL overdosage. The primary anticipated complications of overdosage would consist of bone marrow suppression, peripheral neurotoxicity, and mucositis. Overdoses in pediatric patients may be associated with acute ethanol toxicity (see **PRECAUTIONS: Pediatric Use**).

DOSAGE AND ADMINISTRATION

Note: Contact of the undiluted concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP [di-(2-ethylhexyl)phthalate], which may be leached from PVC infusion bags or sets, diluted TAXOL (paclitaxel) injection solutions should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

All patients should be premedicated prior to TAXOL administration in order to prevent severe hypersensitivity reactions. Such premedication may consist of dexamethasone 20 mg PO administered approximately 12 and 6 hours before TAXOL, diphenhydramine (or its equivalent) 50 mg IV 30 to 60 minutes prior to TAXOL, and cimetidine (300 mg) or ranitidine (50 mg) IV 30 to 60 minutes before TAXOL.

For patients with **carcinoma of the ovary,** the following regimens are recommended (see **CLINICAL STUDIES: Ovarian Carcinoma**):

1) For previously untreated patients with carcinoma of the ovary, one of the following recommended regimens may be given every 3 weeks. In selecting the appropriate regimen, differences in toxicities should be considered (see Table 11 in **ADVERSE REACTIONS: Disease-Specific Adverse Event Experiences**).
   a. TAXOL administered intravenously over 3 hours at a dose of 175 mg/m² followed by cisplatin at a dose of 75 mg/m²; or
   b. TAXOL administered intravenously over 24 hours at a dose of 135 mg/m² followed by cisplatin at a dose of 75 mg/m².
2) In patients previously treated with chemotherapy for carcinoma of the ovary, TAXOL (paclitaxel) Injection has been used at several doses and schedules; however, the optimal regimen is not yet clear. The recommended regimen is TAXOL 135 mg/m² or 175 mg/m² administered intravenously over 3 hours every 3 weeks.

For patients with **carcinoma of the breast,** the following regimens are recommended (see **CLINICAL STUDIES: Breast Carcinoma**):

1) For the adjuvant treatment of node-positive breast cancer, the recommended regimen is TAXOL, at a dose of 175 mg/m² intravenously over 3 hours every 3 weeks for four courses administered sequentially to doxorubicin-containing combination chemotherapy. The clinical trial used four courses of doxorubicin and cyclophosphamide (See **CLINICAL STUDIES: Breast Carcinoma**).
2) After failure of initial chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy, TAXOL at a dose of 175 mg/m² administered intravenously over 3 hours every 3 weeks has been shown to be effective.

For patients with **non-small cell lung carcinoma**, the recommended regimen, given every 3 weeks, is TAXOL administered intravenously over 24 hours at a dose of 135 mg/m² followed by cisplatin, 75 mg/m².

For patients with **AIDS-related Kaposi's sarcoma**,TAXOL administered at a dose of 135 mg/m² given intravenously over 3 hours every 3 weeks or at a dose of 100 mg/m² given intravenously over 3 hours every 2 weeks is recommended (dose intensity 45-50 mg/m²/week). In the two clinical trials evaluating these schedules (see **CLINICAL STUDIES: AIDS-Related Kaposi's Sarcoma**), the former schedule (135 mg/m² every 3 weeks) was more toxic than the latter. In addition, all patients with low performance status were treated with the latter schedule (100 mg/m² every 2 weeks).

Based upon the immunosuppression in patients with advanced HIV disease, the following modifications are recommended in these patients:

1) Reduce the dose of dexamethasone as one of the three premedication drugs to 10 mg PO (instead of 20 mg PO);
2) Initiate or repeat treatment with TAXOL only if the neutrophil count is at least 1000 cells/mm³;
3) Reduce the dose of subsequent courses of TAXOL by 20% for patients who experience severe neutropenia (neutrophil <500 cells/mm³ for a week or longer); and
4) Initiate concomitant hematopoietic growth factor (G-CSF) as clinically indicated.

For the therapy of patients with solid tumors (ovary, breast, and NSCLC), courses of TAXOL should not be repeated until the neutrophil count is at least 1,500 cells/mm³ and the platelet count is at least 100,000 cells/mm³. TAXOL should not be given to patients with AIDS-related Kaposi's sarcoma if the baseline or subsequent neutrophil count is less than 1000 cells/mm³. Patients who experience severe neutropenia (neutrophil <500 cells/mm³ for a week or longer) or severe peripheral neuropathy during TAXOL therapy should have dosage reduced by 20% for subsequent courses of TAXOL. The incidence of neurotoxicity and the severity of neutropenia increase with dose.

Hepatic Impairment: Patients with hepatic impairment may be at increased risk of toxicity, particularly grade III-IV myelosuppression (see **CLINICAL PHARMACOLOGY** and **PRECAUTIONS: Hepatic**). Recommendations for dosage adjustment for the first course of therapy are shown in Table 17 for both 3- and 24-hour infusions. Further dose reduction in subsequent courses should be based on individual tolerance. Patients should be monitored closely for the development of profound myelosuppression.

Table 17: Recommendations for Dosing in Patients With Hepatic Impairment Based on Clinical Trial Data[a]

Degree of Hepatic Impairment			
Transaminase Levels		Bilirubin Levels[b]	Recommended TAXOL Dose[c]
24-hour infusion			
<2 x ULN	and	≤ 1.5 mg/dL	135 mg/m²
2 - <10 x ULN	and	≤ 1.5 mg/dL	100 mg/m²
<10 x ULN	and	1.6 - 7.5 mg/dL	50 mg/m²
≥10 x ULN	or	> 7.5 mg/dL	Not recommended
3-hour infusion			
<10 x ULN	and	≤ 1.25 x ULN	175 mg/m²
<10 x ULN	and	1.26 - 2.0 x ULN	135 mg/m²
<10 x ULN	and	2.01 - 5.0 x ULN	90 mg/m²
≥10 x ULN	or	> 5.0 x ULN	Not recommended

[a] These recommendations are based on dosages for patients without hepatic impairment of 135 mg/m² over 24 hours or 175 mg/m² over 3 hours; data are not available to make dose adjustment recommendations for other regimens (eg, for AIDS-related Kaposi's sarcoma).

[b] Differences in criteria for bilirubin levels between the 3- and 24-hour infusion are due to differences in clinical trial design.

[c] Dosage recommendations are for the first course of therapy; further dose reduction in subsequent courses should be based on individual tolerance.

Preparation and Administration Precautions: TAXOL is a cytotoxic anticancer drug and, as with other potentially toxic compounds, caution should be exercised in handling TAXOL. The use of gloves is recommended. If TAXOL solution contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure, events have included tingling, burning, and redness. If TAXOL contacts mucous membranes, the membranes should be flushed thoroughly with water. Upon inhalation, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported.

Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. (See **PRECAUTIONS: Injection Site Reaction.**)

Preparation for Intravenous Administration: TAXOL must be diluted prior to infusion. TAXOL should be diluted in 0.9% Sodium Chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP, or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. The solutions are physically and chemically stable for up to 27 hours at ambient temperature (approximately 25°C) and room lighting conditions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

Chemo Dispensing Pin™ is a trademark of B. Braun Medical Incorporated.

Upon preparation, solutions may show haziness, which is attributed to the formulation vehicle. No significant losses in potency have been noted following simulated delivery of the solution through IV tubing containing an in-line (0.22 micron) filter.

Data collected for the presence of the extractable plasticizer DEHP [di-(2-ethylhexyl)phthalate] show that levels increase with time and concentration when dilutions are prepared in PVC containers. Consequently, the use of plasticized PVC containers and administration sets is not recommended. TAXOL solutions should be prepared and stored in glass, polypropylene, or polyolefin containers. Non-PVC containing administration sets, such as those which are polyethylene-lined, should be used.

TAXOL should be administered through an in-line filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® filters which incorporate short inlet and outlet PVC-coated tubing has not resulted in significant leaching of DEHP.

The Chemo Dispensing Pin™ device or similar devices with spikes should not be used with vials of TAXOL since they can cause the stopper to collapse resulting in loss of sterile integrity of the TAXOL solution.

Stability: Unopened vials of TAXOL (paclitaxel) Injection are stable until the date indicated on the package when stored between 20°-25° C (68°-77°F), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product. Upon refrigeration components in the TAXOL vial may precipitate, but will redissolve upon reaching room temperature with little or no agitation. There is no impact on product quality under these circumstances. If the solution remains cloudy or if an insoluble precipitate is noted, the vial should be discarded. Solutions for infusion prepared as recommended are stable at ambient temperature (approximately 25°C) and lighting conditions for up to 27 hours.

HOW SUPPLIED

NDC 0015-3475-30	30 mg/5 mL multidose vial individually packaged in a carton.
NDC 0015-3476-30	100 mg/16.7 mL multidose vial individually packaged in a carton.
NDC 0015-3479-11	300 mg/50 mL multidose vial individually packaged in a carton.

Storage: Store the vials in original cartons between 20°-25°C (68°-77°F). Retain in the original package to protect from light.

Handling and Disposal: Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-7] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

REFERENCES

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2. AMA Council Report. Guidelines for Handling Parenteral Antineoplastics. *JAMA* 1985; 253(11):1590-1592.
3. National Study Commission on Cytotoxic Exposure–Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts, 02115.
4. Clinical Oncological Society of Australia. Guidelines and Recommendations for Safe Handling of Antineoplastic Agents. *Med J Australia* 1983; 1:426-428.
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7. Controlling Occupational Exposure to Hazardous Drugs. (OSHA WORK-PRACTICE GUIDELINES.) *Am J Health-Syst Pharm* 1996; 53:1669-1685.

BRISTOL-MYERS SQUIBB ONCOLOGY

Bristol-Myers Squibb Company
Princeton, NJ 08543
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℞ ONLY

PATIENT INFORMATION *TAXOL*® INJECTION
(generic name = paclitaxel)

WHAT IS TAXOL?
TAXOL is a prescription cancer medicine. It is injected into a vein and it is used to treat different types of tumors. The tumors include advanced ovary and breast cancer. The tumors also include certain lung cancers (non-small cell) in people who cannot have surgery or radiation therapy. TAXOL may also be used to treat AIDS-related Kaposi's sarcoma.

WHAT IS CANCER?
Under normal conditions, the cells in your body divide and grow in an orderly, controlled way. Cell division and growth are necessary for the human body to perform its functions and to repair itself, when necessary. Cancer cells are different from normal cells because they are not able to control their own growth. The reasons for this abnormal growth are not yet fully understood.

A tumor is a mass of unhealthy cells that are dividing and growing fast and in an uncontrolled way. When a tumor invades surrounding healthy body tissue it is known as a malignant tumor. A malignant tumor can spread (metastasize) from its original site to other parts of the body if not found and treated early.

HOW DOES TAXOL WORK?
TAXOL is a type of medical treatment called chemotherapy. The purpose of chemotherapy is to kill cancer cells or prevent their growth.

All cells, whether they are healthy cells or cancer cells, go through several stages of growth. During one of the stages, the cell starts to divide. TAXOL may stop the cells from dividing and growing, so they eventually die. In addition, normal cells may also be affected by TAXOL causing some of the side effects. (See **WHAT ARE THE POSSIBLE SIDE EFFECTS OF TAXOL?** below.)

WHO SHOULD NOT TAKE TAXOL?
Patients who have a history of hypersensitivity (allergic reactions) to TAXOL or other drugs containing Cremophor® EL* (polyoxyethylated castor oil), like cyclosporine or teniposide, should not be given TAXOL. In addition, TAXOL should not be given to patients with dangerously low white blood cell counts.

HOW IS TAXOL (PACLITAXEL) INJECTION GIVEN?
TAXOL is injected into a vein (intravenous (I.V.) infusion). Before you are given TAXOL, you will have to take certain medicines (premedications) to prevent or reduce the chance you will have a serious allergic reaction. Such reactions have occurred in a small number of patients while receiving TAXOL and have been rarely fatal. (See **WHAT ARE THE POSSIBLE SIDE EFFECTS OF TAXOL?** below).

WHAT ARE THE POSSIBLE SIDE EFFECTS OF TAXOL?
Most patients taking TAXOL will experience side effects, although it is not always possible to tell whether such effects are caused by TAXOL, another medicine they may be taking, or the cancer itself. Important side effects are described below; however, some patients may experience other side effects that are less common. *Report any unusual symptoms to your doctor.*

Important side effects observed in studies of patients taking TAXOL were as follows:

- *Allergic reactions.* Allergic reactions can vary in degrees of severity. They may cause death in rare cases. When a severe allergic reaction develops, it usually occurs at the time the medicine is entering the body (during TAXOL infusion). Allergic reactions may cause trouble breathing, very low blood pressure, sudden swelling, and/or hives or rash. The likelihood of a serious allergic reaction is lowered by the use of several kinds of medicines that are given to you before the TAXOL (paclitaxel) Injection infusion.
- *Heart and blood vessel (cardiovascular) effects.* TAXOL may cause a drop in heart rate (bradycardia) and low blood pressure (hypotension). The patient usually does not notice these changes. These changes usually do not require treatment. Your heart function, including blood pressure and pulse, will be monitored while you are receiving the medicine. You should notify your doctor if you have a history of heart disease.
- *Infections due to low white blood cell count.* Among the body's defenses against bacterial infections are white blood cells. Between your TAXOL treatment cycles, you will often have blood tests to check your white blood cell counts. TAXOL usually causes a brief drop in white blood cells. *If you have a fever (temperature above 100.4° F) or other sign of infection, tell your doctor right away. Sometimes serious infections develop that require treatment in the hospital with antibiotics. Serious illness or death could result if such infections are not treated when white blood cell counts are low.*
- *Hair loss.* Complete hair loss, or alopecia, almost always occurs with TAXOL. This usually involves the loss of eyebrows, eyelashes, and pubic hair, as well as scalp hair. It can occur suddenly after treatment has begun, but usually happens 14 to 21 days after treatment. *Hair generally grows back after you've finished your TAXOL treatment.*
- *Joint and muscle pain.* You may get joint and muscle pain a few days after your TAXOL treatment. These symptoms usually disappear in a few days. Although pain medicine may not be necessary, tell your doctor if you are uncomfortable.
- *Irritation at the injection site.* TAXOL sometimes causes irritation at the site where it enters the vein. Reactions may include discomfort, redness, swelling, inflammation (of the surrounding skin or of the vein itself), and ulceration (open sores). These reactions are usually caused by the I.V. (intravenous) fluid leaking into the surrounding area. *If you notice anything unusual at the site of the injection (needle), either during or after treatment, tell your doctor right away.*
- *Low red blood cell count.* Red blood cells deliver oxygen to tissues throughout all parts of the body and take carbon dioxide from the tissues by using a protein called hemoglobin. A lowering of the volume of red blood cells may occur following TAXOL treatment causing anemia. Some patients may need a blood transfusion to treat the anemia.

  Patients can feel tired, tire easily, appear pale, and become short of breath. Contact your doctor if you experience any of these symptoms following TAXOL treatment.
- *Mouth or lip sores (mucositis).* Some patients develop redness and/or sores in the mouth or on the lips. These symptoms might occur a few days after the TAXOL treatment and usually decrease or disappear within one week. Talk with your doctor about proper mouth care and other ways to prevent or reduce your chances of developing mucositis.
- *Numbness, tingling, or burning in the hands and/or feet (neuropathy).* These symptoms occur often with TAXOL and usually get better or go away without medication within several months of completing treatment. However, if you are uncomfortable, tell your doctor so that he/she can decide the best approach for relief of your symptoms.
- *Stomach upset and diarrhea.* Some patients experience nausea, vomiting, and/or diarrhea following TAXOL use. If you experience nausea or stomach upset, tell your doctor. Diarrhea will usually disappear without treatment; however, *if you experience severe abdominal or stomach area pain and/or severe diarrhea, tell your doctor right away.*

Talk with your doctor or other healthcare professional to discuss ways to prevent or reduce some of these side effects. Because this leaflet does not include all possible side effects that can occur with TAXOL, it is important to talk with your doctor about other possible side effects.

CAN I TAKE TAXOL IF I AM PREGNANT OR NURSING A BABY?
TAXOL could harm the fetus when given to a pregnant woman. Women should avoid becoming pregnant while they are undergoing treatment with TAXOL. *Tell your doctor if you become pregnant or plan to become pregnant while taking TAXOL.*

Because studies have shown TAXOL to be present in the breast milk of animals receiving the drug, it may be present in human breast milk as well. Therefore, nursing a baby while taking TAXOL is NOT recommended.

This medicine was prescribed for your particular condition. This summary does not include everything there is to know about TAXOL. Medicines are sometimes prescribed for purposes other than those listed in a Patient Information Leaflet. If you have questions or concerns, or want more information about TAXOL, your doctor and pharmacist have the complete prescribing information upon which this guide is based. You may want to read it and discuss it with your doctor. Remember, no written summary can replace careful discussion with your doctor.

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